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GELS FOR ENCAPSULATION OF BIOLOGICAL MATERIALS

This application is a Continuation of 08/510,089, filed

August 1, 1995, which is a CIP of 07/958,870, filed 10/7/92,

15   which is a CIP of 07/870,540 (abandoned), filed 4/20/92; this is

also a CIP of 08/379,848, filed 1/27/95, which is a continuation

of 08/022,687 filed 3/1/93 and issued as U.S.P.N. 5,410,016,

which is a CIP of 07/843,485, filed 2/28/92 (abandoned); this is

also a CIP of 08/336,393, filed 11/10/94, which is a

20   continuation of 07/598,880, filed 10/15/90 (abandoned.)

BACKGROUND

Microencapsulation technology holds promise in many areas

of medicine. For example, some important applications are

treatment of diabetes (Goosen, M. F. A., et al. (1985)

25   Biotechnology and Bioengineering, 27:146.), production of

biologically important chemicals (Omata, T., et al. (1979)

European J. Appl. Microbiol. Biotechnol., 6:207:215), evaluation

of anti-human immuno-deficiency virus drugs (McMahon, J., et

al., (1990) J. Nat. Cancer Inst., 82(22) 1761-1765),

30   encapsulation of hemoglobin for red blood cell substitutes, and

controlled release of drugs. During encapsulation using prior

methods, cells are often exposed to processing conditions which

are potentially cytotoxic. These conditions include heat,

organic solvents and

35

5   non-physiological pH which can kill or functionally impair cells.

Proteins are often exposed to conditions which are potentially

denaturing and can result in loss of biological activity.

Further, even if cells survive processing conditions,

the stringent requirements of encapsulating polymers for

10   biocompatibility, chemical stability, immunoprotection and

resistance to cellular overgrowth, restrict the applicability of

prior art methods. For example, the encapsulating method based

on ionic crosslinking of alginate (a polyanion) with polylysine

or polyornithine (polycation) (Goosen, M. F. A., et al. (1985)

15   Biotechnology and Bioengineering, 27:146) offers relatively mild

encapsulating conditions, but the long-term mechanical and

chemical stability of such ionically crosslinked polymers remains

doubtful. Moreover, these polymers when implanted in vivo, are

susceptible to cellular overgrowth (McMahon, J., et al. (1990) J.

20   Nat. Cancer Inst., 82(22) 1761-1765) which restricts the

permeability of the microcapsule to nutrients, metabolites, and

transport proteins from the surroundings. This has been seen to

possibly lead to starvation and death of encapsulated islets of

Langerhans cells (O'Shea, G. M. et al. (1986) Diabetes, 35:943-

25   946).

Thus, there is a need for a relatively mild cell

encapsulation method which offers control over properties of the

encapsulating polymer. The membranes must be non-toxically

produced in the presence of cells, with the qualities of being

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5   permselective, chemically stable, and very highly biocompatible.

A similar need exists for the encapsulation of biological

materials other than cells and tissues.

Biocompatibility

       Materials are considered biocompatible if the material

10   elicits either a reduced specific humoral or cellular immune

response or does not elicit a nonspecific foreign body response

that prevents the material from performing the intended function,

and if the material is not toxic upon ingestion or implantation.

The material also must not elicit a specific reaction such as

15   thrombosis if in contact with the blood.

Use of Gels in Biomaterials

       Gels made of polymers which swell in water such as poly

(HEMA), water-insoluble polyacrylates, and agarose, have been

shown to be capable of encapsulating islet cells and other animal

20   tissue (Iwata, H., et al. (1989) Diabetes, 38:224-225; Lambert,

F. V., et al. (1984) Appl. Biochem. Biotech, 10:101-105).

However, these gels have undesirable mechanical properties.

Agarose forms a weak gel, and the polyacrylates must be

precipitated from organic solvents, thus increasing the potential

25   for cytotoxicity. Dupuy et al. ((1988) J. Biomed. Mater. Res.,

22:1061-1070) have reported the microencapsulation of islets by

polymerization of acrylamide to form polyacrylamide gels.

However, the polymerization process, if allowed to proceed

rapidly to completion, generates local heat and requires the

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5   presence of toxic cross-linkers. This usually results in

mechanically weak gels whose immunoprotective ability has not

been established. Moreover, the presence of a low molecular

weight monomer is required which itself is cytotoxic.

Microcapsules formed by the coacervation of alginate

10   and poly(L-lysine) have been shown to be immunoprotective e.g.,

O'Shea, G. M. et al. (1986) Diabetes, 35:943-946. However,

implantation for periods up to a week has resulted in severe

fibrous overgrowth on these microcapsules (McMahon, J., et al.

(1990) J. Nat. Cancer Inst., 82(22) 1761-1765; O'Shea, G. M. et

15   al. (1986) Diabetes, 35:943-946).

Synthetic Biodegradable Polymers

The field of biodegradable polymers has developed

rapidly since the synthesis and biodegradability of polylactic

acid was first reported by Kulkarni et al., (1966) Arch. Surg.,

20   93:839. Several other polymers are known to biodegrade,

including polyanhydrides and polyorthoesters, which take

advantage of labile backbone linkages, as reported by Domb et

al., 1989 Macromolecules, 22:3200; Heller et al., 1990

BIODEGRADABLE POLYMERS AS DRUG DELIVERY SYSTEMS, Chasin, M. and

25   Langer, R., Eds., Dekker, New York, 121-161. Since it is

desirable to have polymers that degrade into naturally occurring

materials, polyaminoacids have been synthesized, as reported by

Miyake et al., (1974), for *in vivo* use. This was the basis for

using polyesters (Holland et al., 1986 Controlled Release, 4:155-

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5   180) of α-hydroxy acids (*viz.*, lactic acid, glycolic acid), which

       remain the most widely used biodegradable materials for

       applications ranging from closure devices (sutures and staples)

       to drug delivery systems (U.S. Patent No. 4,741,337 to Smith et

       al.; Spilizewski et al., (1985) J. Control. Rel. 2:197-203).

10   The time required for a polymer to degrade can be

       tailored by selecting appropriate monomers. Differences in

       crystallinity also alter degradation rates. Due to the

       relatively hydrophobic nature of these polymers, actual mass loss

       only begins when the oligomeric fragments are small enough to be

15   water soluble. Hence, initial polymer molecular weight

       influences the degradation rate.

              Degradable polymers containing water-soluble polymer

       elements have been described. Sawhney et al., (1990) J. Biomed.

       Mater. Res. 24:1397-1411, copolymerized lactide, glycolide and ε-

20   caprolactone with PEG to increase its hydrophilicity and

       degradation rate. U.S. Patent No. 4,716,203 to Casey et al.

       (1987) synthesized a PGA-PEG-PGA block copolymer, with PEG

       content ranging from 5-25% by mass. U.S. Patent No. 4,716,203 to

       Casey et al. (1987) also reports synthesis of PGA-PEG diblock

25   copolymers, again with PEG ranging from 5-25%. U.S. Patent No.

       4,526,938 to Churchill et al. (1985) described noncrosslinked

       materials with MW in excess of 5,000, based on similar

       compositions with PEG; although these materials are not water

       soluble. Cohn et al. (1988) J. Biomed. Mater. Res. 22:993-1009

5   described PLA-PEG copolymers that swell in water up to 60%; these

polymers also are not soluble in water, and are not crosslinked.

These materials all use both water-soluble polymers and

degradable polymers, and they are all insoluble in water,

collectively swelling up to about 60%.

10   Degradable materials of biological origin are well

known, for example, crosslinked gelatin. Hyaluronic acid has

been crosslinked and used as a degradable swelling polymer for

biomedical applications (U.S. Patent No. 4,987,744 to Della Valle

et al., U.S. Patent 4,957,744 to Della Valle et al. (1991) Polym.

15   Mater. Sci. Eng., 62:731-735).

<u>Use of Biodegradable Materials for Controlled Drug Release</u>

Most hydrophilic drugs are mechanically dispersed as

suspensions within solutions of biodegradable polymers in organic

solvents. Protein and enzyme molecular conformations are

20   frequently different under these circumstances than they would be

in aqueous media. An enzyme dispersed in such a hydrophobic

matrix is usually present in an inactive conformation until it is

released into the surrounding aqueous environment subsequent to

polymer degradation. Additionally, some proteins may be

25   irreversibly denatured by contact with organic solvents used in

dispersing the protein within the polymer.

<u>Use of PEO in Biomaterials</u>

The use of poly(ethylene oxide) (PEO) to increase

biocompatibility is well documented in the literature. The

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presence of grafted PEO on the surface of bovine serum albumin

has been shown by Abuchowski, A. et al. ((1977) J. Biol. Chem.,

252:3578) to reduce immunogenicity in a rabbit and to increase

circulation times of exogenous proteins in animals. The

biocompatibility of algin-poly(L-lysine) microcapsules has been

significantly enhanced by incorporating a graft copolymer of PLL

and PEO on the microcapsule surface (Sawhney, et al.,

Biomaterials 13:863-870 (1991)).

The grafting of methoxy PEO onto polyacrylonitrile

surfaces was seen by Miyama et al. ((1988) J. Appl. Polym. Sci.,

35:115-125) to render the polyacrylonitrile surface relatively

non-thrombogenic. Nagoaka et al. (Polymers as Biomaterials,

Shalaby, S. W. ed., Plenum Press, New York) synthesized a graft

copolymer of methacrylates with PEO and found the resulting

polymer to be highly non-thrombogenic. Desai and Hubbell have

immobilized PEO on poly(ethylene terepthalate) surfaces by

forming a physical interpenetrating network (Desai et al., (1992)

Macromolecules 25:226); they have shown these surfaces to be

highly resistant to thrombosis (Desai et al, (1991) Biomaterials,

12:144) and to both mammalian and bacterial cell growth (Desai,

et al., submitted).

PEO is a unique polymer in terms of structure. The PEO

chain is highly water soluble and highly flexible. PEO chains

have an extremely high motility in water and are essentially non-

ionic in structure. The synthesis and characterization of PEO

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5   derivatives which can be used for attachment of PEO to various

surfaces, proteins, drugs etc. has been reviewed (Harris, (1985)

JNS-Rev. Macromol. Chem. Phys., C25:325-373). Other polymers are

also water soluble and non-ionic, such as poly(N-vinyl

pyrrolidinone) and poly(ethyl oxazoline). These have been used

10   to reduce interaction of cells with tissues. Desai et al. (1991)

Biomaterials, 12:144. Water soluble ionic polymers, such as

hyaluronic acid, have also been used to reduce cell adhesion to

surfaces and can similarly be used.

        Electron beam cross-linking has been used to synthesize

15   PEO hydrogels, and these biomaterials have been reported to be

non-thrombogenic (Sun, et al., (1987) Polymer Prepr., 28:292-294;

Dennison, H.A., (1986) Ph.D. Thesis. Massachusetts Institute of

Technology). However, use of an electron beam precludes the

presence of any living tissue due to the sterilizing effect of

20   this radiation. Also, the networks produced are difficult to

characterize due to the non-specific cross-linking induced by the

electron beam.

        Photopolymerizable PEG diacrylates have been used to

entrap yeast cells for fermentation and chemical conversion

25   (Kimura et al. (1981) Eur. J. Appl. Microbio. Biotechnol., 11:78-

80; Omata et al., (1981) Eur. J. Appl. Microbial Biotechnol.,

11:199-204; Okada et al. (1987) Appl. Microbiol. Biotechnol.,

26:112-116). Other methods for encapsulation of cells within

materials photopolymerizable with short wavelength ultraviolet

5   radiation have been used with microbial cells (Tanaka, et al.,

(1977) Eur. J. Biochem, 80:193-197; Omata, et al., (1979)

European J. Appl. Microbiol. Biotechnol., 6:207-215; Omata, et

al., (1979) Eur. J. Appl. Microhiol. Biotechnol. 8:143-155; Chun,

et al., (1981)J. Gen. App. Microbiol., 27:505-509; Fukui, et al.,

10 (1976) Febs Letters, 66:2; Fukui, et al., (1984) Advances in

Biochemical Engineering and Biotechnology, 29:1-33).   However,

yeast cells and some microbial cells are much hardier and

resistant to adverse environments, elevated temperatures, and

short wavelength ultraviolet radiation than mammalian cells and

15 human tissues.

There are several problems with these methods,

including the use of methods and/or materials which are

thrombogenic or unstable *in vivo*, or require polymerization

conditions which tend to destroy living mammalian tissue or

20 biologically active molecules, for example, short wavelength

ultraviolet radiation.   In order to encapsulate living tissue for

implantation in a human or other mammalian subject, the

polymerization conditions must not destroy the living tissue, and

the resulting polymer-coated cells must be biocompatible.

25 There is also a need to encapsulate materials within a

very thin layer of material that is permeable to nutrients and

gases, yet strong and non-immunogenic.   For example, for

transplantation of islets of Langerhans, the islets, which have a

diameter of 100 to 200 microns, have in the past been

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5   encapsulated within microspheres that have a diameter of 400 to

1000 microns. This large diameter can result in slowed diffusion

of nutritional molecules and large transplantation volumes.

In summary, there is a need for materials, and methods

of use thereof, which can be used to encapsulate cells and

10   tissues or biologically active molecules which are biocompatible,

do not elicit specific or non-specific immune responses, and

which can be polymerized in contact with living cells or tissue

without injuring or killing the cells, within a very short time

frame, and in a very thin layer. An important aspect of the use

15   of these materials *in vivo* is that they must be polymerizable

within the time of a short surgical procedure or before the

material to be encapsulated disperses, is damaged or dies.

It is therefore an object of the present invention to

provide a polymeric material that can be polymerized in contact

20   with living cells and tissues, and in a very short time period.

It is a further object of the present invention to

provide a polymeric material which is biocompatible and resistant

to degradation for a specific time period.

It is a still further object of the present invention

25   to provide a polymeric material which is permeable to nutrients

and gases yet can protect cells and tissues from *in vivo* attack

by other cells.

5   It is yet a further object of the present invention to

provide implantable biodegradable materials and biodegradable

materials for encapsulation of cells and tissue.

           It is another object of the present invention to

provide biogels which can be both ionically and covalently

10   crosslinked.

           It is an additional object of the present invention to

provide a crosslinked biocompatible material which has at least

one ionically crosslinked component and at least one covalently

crosslinked component.

15   **SUMMARY OF THE INVENTION**

           This invention provides novel methods for the formation

of biocompatible membranes around biological materials using

photopolymerization of water soluble molecules. The membranes

can be used as a covering to encapsulate biological materials or

20   biomedical devices, as a "glue" to cause more than one biological

substance to adhere together, or as carriers for biologically

active species.

           Several methods for forming these membranes are

provided. Each of these methods utilizes a polymerization system

25   containing water-soluble macromers, species which are at once

polymers and macromolecules capable of further polymerization.

The macromers are polymerized using a photoinitiator (such as a

dye), optionally a cocatalyst, optionally an accelerator, and

5   radiation in the form of visible or long wavelength UV light.
       The reaction occurs either by suspension polymerization or by
       interfacial polymerization. The polymer membrane can be formed
       directly on the surface of the biological material, or it can be
       formed on material which is already encapsulated.

10   The macromers, which are water soluble or substantially
       water soluble, are too large to diffuse into the cells to be
       coated. Examples of macromers include highly biocompatible PEG
       hydrogels, which can be rapidly formed in the presence or absence
       of oxygen, without use of toxic polymerization initiators, at

15   room or physiological temperatures, and at physiological pH.

               Some macromers of this invention include at least one
       water soluble region, at least one region which is biodegradable,
       usually by hydrolysis, and at least two free radical-
       polymerizable regions. The regions can, in some embodiments, be

20   both water soluble and biodegradable.

               Ultrathin membranes can be formed by the methods
       described herein. These ultrathin membranes allow for optimal
       diffusion of nutrient and bioregulator molecules across the
       membrane, and great flexibility in the shape of the membrane.

25   Such thin membranes produce encapsulated material with optimal
       economy of volume. Biological material thus coated can be packed
       into a relatively small space without interference from bulky
       membranes.

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5   The thickness and pore size of membranes formed can be

varied. This variability allows for "perm-selectivity" --

membranes can be adjusted to the desired degree of porosity,

allowing only preferred molecules to permeate the membrane, while

acting as a barrier against larger undesired molecules. Thus,

10   the membranes are immunoprotective in that they prevent the

transfer of antibodies or cells of the immune system.

         When the encapsulated biological material is cellular

in nature, the absence of small monomers in the polymerization

solution prevents the diffusion of toxic molecules into the cell.

15   In this manner the present invention provides a polymerization

system which is more biocompatible than any available in the

prior art.

         Additionally, the polymerization method can utilize

short bursts of visible or long wavelength UV light which is

20   nontoxic to biological material. Bioincompatible polymerization

initiators employed in the prior art are also eliminated.

         According to the present invention, membrane formation

occurs under physiological conditions. Thus, no damage is done

to the enclosed biological material due to harsh pH, temperature,

25   or ionic conditions.

         Because the membrane adheres to the biological materi-

al, the membrane can be used as an adhesive to fasten more than·

one biological substance together. The macromers are polymerized

in the presence of these substances which are in close proximity.

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5   The membrane forms in the interstices, effectively gluing the

substances together.

     Additionally, utilizing the tendency of the membrane to

adhere to biological material, a membrane can be formed around or

on a biologically active substance to act as a carrier for that

10   substance.

BRIEF DESCRIPTION OF THE FIGURES

     Figure 1 shows schematically illustrated macromers of the

present invention where _____ is a water soluble core such as

PEG; ~~~~~ is a hydrolyzably degradable extension such as a

15   polyglycolide; ====== is a polymerizable end cap or side chain

such as an acrylate; and ------ is a water-soluble and

hydrolyzable portion such as a hyaluronate.

     Figure 2A is a schematic of dye-initiated polymerization of

a PEG layer around crosslinked alginate microspheres.

20   Figure 2B is a photomicrograph of the alginate/poly(L-

lysine) microspheres containing human islets of Langerhans coated

with a PEG 18.5K tetraacrylate hydrogel using the dye binding

method depicted in Figure 2A.

     Figure 3 is a schematic of photopolymerization of a PEG

25   coating on alginate-poly(L-lysine) microspheres suspended in

mineral oil.

5   Figure 4 is a photomicrograph of Islets of Langerhans

isolated from a human pancreas encapsulated in a PEG 18.5K

tetraacrylate hydrogel.

Figure 5 is a schematic representation of coextrusion

apparatus used for microencapsulation using laser polymerization

10   Figure 6 is a photomicrograph of microspheres produced by

laser polymerization of PEG 400 diacrylate around cells.

Figure 7A is a photomicrograph of alginate-PLL microspheres

recovered after 4 days following implantation i.p. in mice.

Figure 7B is a photomicrograph of Alginate-PLL microspheres

15   coated with a PEG 18.5K Da tetraacrylate, using the dye diffusion

method depicted in Figure 1.

Figures 8A-F is a graph of the number of cells versus gel

composition, for the unattached cells obtained from lavage of the

peritoneal cavity in mice with different PEO overcoat gel

20   compositions: a - 18.5k; b - 10% 0.5k, 90% 18.5k; c - 50% 18.5k,

50% 0.4k; d - 10% 0.4k, 90% 35k; e - 50% 0.4k, 50% 35k; and f -

alginate-poly(L-lysine) control.

Figure 9 is a graph of the % protein released versus time in

minutes, for diffusion of bovine serum albumin (open squares),

25   human IgG (triangles) and human fibrinogen (closed squares)

through a PEO 18.5K-tetraacrylate gel.

Figure 10 is a graph of the % diffusion of bovine serum

albumin over time in minutes through PEO 400 diacrylate (open

squares) and PEG 18.5K-tetracrylate (triangles) gels.

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5   Figure 11A is a graph of the length in mm of gel produced by

argon ion laser induced polymerization versus log (time) (ms) of

trimethylolpropane using an amine and ethyl eosin initiation

system.

       Figure 11B is a photomicrograph of the spikes formed as a

10   result of laser irradiation of ethoxylated trimethylol propane

triacrylate for durations of 67 ms, 125 ms, 250 ms, 500 ms, and 1

sec.

       Figure 12A is a photomicrograph of human foreskin

fibroblasts cultured for 6 h on a glass coverslip coated with PEG

15   18.5K-tetraacrylate gel.

       Figure 12B is a photomicrograph of human foreskin

fibroblasts cultured for 6 h on a glass that was not coated with

PEG.

       Figure 13 is a photomicrograph of PEG 18.5K-tetraacrylate

20   microspherical gels, implanted in mice, and explanted after 4

days, showing very little fibrous overgrowth.

       Figures 14A-C are creep curves for PEG diacrylate and

tetraacrylate gels; test and recovery loads are given below the

abscissa: A - 1k; B - 6K; and C - 18.5K PEG gels.

25   Figure 15 shows the degree of photopolymerization (dp)

calculated and found by NMR.

       Figure 16A shows Human foreskin fibroblasts cultured for six

hours on glass coverslips coated with PEG 18.5K-glycolide

diacrylate (18.5KG).

5   Figure 16B shows Human foreskin fibroblasts cultured for six

hours on glass coverslips not coated with PEG.

Figure 17A shows the release of BSA from a PEG 1K (1000

molecular weight PEG) glycolide diacrylate with glycolide

extensions (1 KG) hydrogel into PBS.

10   Figure 17B shows release of lysozyme from PEG 18.5K-DL-

lactide tretraacrylate (18.5KL) into PBS.

Figure 18A shows release of active recombinant tPA from a

PEG 1K lactide diacrylate (1KL) hydrogel.

Figure 18B shows release of active recombinant t-PA from PEG

15   4K glycolide diacrylate (4KG) hydrogel.

Figure 18C shows release of active recombinant tPA from a

PEG 18.5K-glycolide diacrylate (18.5KG) hydrogel into PBS.

Figure 19A is a superior view of rabbit uterine horn used as

a control. Distorted horn anatomy with 66% adhesions is evident.

20   The horns are folded upon themselves.

Figure 19B is a superior view of rabbit uterine horn treated

with a photopolymerized biodegradable hydrogel, PEG 18.5KL. Horn

anatomy is normal, with no adhesion bands visible.

Figure 20A is an environmental scanning electron micrograph

25   (ESEM) of an untreated blood vessel following trauma.

Figure 20B is an ESEM of a polymer coated blood vessel

following trauma.

DETAILED DESCRIPTION

5   By a variety of methods, this invention provides a

means for creating biocompatible membranes of varying thickness

on the surface of a variety of biological materials. The

polymerization occurs by a free-radical reaction, causing a

10   "macromer" with at least two ethylenically unsaturated moieties

to form a crosslinked polymer. The components of this reaction

are:

        (1)   a photoinitiator, preferably eosin dye;

        (2)   a "macromer," preferably polyethylene glycol (PEG)

15   diacrylate, m.w. 18.5 kD. This component is at

               once a polymer and a macromolecule capable of

               further polymerization;

        (3)   optionally a cocatalyst, preferably

               triethanolamine; and

20   (4)   optionally, an accelerator.

These components are mixed in varying combinations, and the

mixture is exposed to longwave UV or visible light ("radiation"),

preferably of wavelength 350-700 nm, most preferred at 365-

514 nm, to initiate polymerization. A network is formed as the

25   macromers polymerize in a variety of directions.

POLYMERIZATION

Four methods are used to effect polymerization to form biocompatible membranes. These are referred to below as the "bulk suspension polymerization" method, the "microcapsule suspension polymerization" method, the "microcapsule interfacial polymerization" method, and the "direct interfacial polymerization" method. They utilize either suspension or interfacial polymerization techniques on either coated or uncoated biological material.

1. BULK SUSPENSION POLYMERIZATION METHOD

In this embodiment of the invention the core biological material is mixed in an aqueous macromer solution (composed of the macromer, cocatalyst and optionally an accelerator) with the photoinitiator. Small globular geometric structures such as spheres, ovoids, or oblongs are formed, preferably either by coextrusion of the aqueous solution with air or with a non-miscible substance such as oil, preferably mineral oil, or by agitation of the aqueous phase in contact with a non-miscible phase such as an oil phase to form small droplets. The macromer in the globules is then polymerized when exposed to radiation. Because the macromer and initiator are confined to the globules, the structure resulting from polymerization is a capsule in which the biological material is enclosed. This is a "suspension polymerization" whereby the entire aqueous portion of the globule

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5 polymerizes to form a thick membrane around the cellular

material.

2. MICROCAPSULE SUSPENSION POLYMERIZATION METHOD

This embodiment of the invention employs microencapsu-

lated material as a core about which the macromer is polymerized

10 in a suspension polymerization reaction. The biological material

is first encapsulated, such as in an alginate microcapsules. The

microcapsule is then mixed as in the first embodiment with the

macromer solution and the photoinitiator, and then polymerized by

radiation. In the event an ionically crosslinkable material such

15 as alginate is used for the first encapsulation, this results in

a biocompatible material which is both ionically crosslinked and

covalently crosslinked.

This method takes advantage of the extreme

hydrophilicity of PEG macromer, and is especially suited for use

20 with hydrogel microcapsules such as alginate-poly(L-lysine). The

microsphere is swollen in water. When a macromer solution (with

the initiating system) is forced to phase separate in a

hydrophobic medium, such as mineral oil, the PEG macromer

solution prefers to stay on the hydrophilic surface of the

25 alginate microcapsule. When this suspension is irradiated, the

PEG macromer undergoes polymerization and gelation, forming a

thin layer of polymeric, water insoluble gel around the

microsphere. Agarose beads have been used in an analogous way by

Gin et al.(1987) J. Microencapsulation, 4:239-242 as scaffolds to

- 20 -

5   carry out polymerization of acrylamide. However, that method is

limited by potential toxicity associated with the use of a low

molecular weight monomer, as opposed to the macromeric precursors

of the present invention.

This technique preferably involves coextrusion of the

10   microcapsule in a solution of macromer and photoinitiator, the

solution being in contact with air or a liquid which is non-

miscible with water, to form droplets which fall to a container

such as a petri dish containing a solution such as mineral oil in

which the droplets are not miscible. The non-miscible liquid is

15   chosen for its ability to maintain droplet formation.

Additionally, if the membrane-encapsulated material is to be

injected or implanted in an animal, any residue should be non-

toxic and non-immunogenic. Mineral oil is a preferred non-

miscible liquid.

20   On the petri dish the droplets are exposed to radiation

which causes polymerization. This coextrusion technique results

in a crosslinked polymer coat of greater than 50 microns

thickness. Alternatively, the microcapsules may be suspended in

a solution of macromer and photoinitiator which is agitated in

25   contact with a non-miscible phase such as an oil phase. The

emulsion which results is irradiated to form a polymer coat,

again of greater than 50 microns thickness.

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5   3. MICROCAPSULE INTERFACIAL POLYMERIZATION METHOD

In this embodiment, the biological material is also microencapsulated as in the previous method. However, rather than suspension polymerization, interfacial polymerization is utilized to form the membrane. This involves coating the

10   microcapsule with photoinitiator, suspending the microcapsule in the macromer solution, and immediately irradiating. By this technique a thin polymer coat, of less than 50 microns thickness, is formed about the microcapsule, because the photoinitiator is present only at the microcapsule surface and is given

15   insufficient time to diffuse far into the macromer solution. As a result, the initiator is present in only a thin shell of the aqueous solution, causing a thin layer to be polymerized.

When the microcapsules are in contact with dye solution, the dye penetrates into the inner core of the

20   microcapsule as well as adsorbing to the surface. When such a microcapsule is put into a solution containing a macromer and, optionally, a cocatalyst such as triethanolamine, and exposed to laser light, initially all the essential components of the reaction are present only at and just inside the interface of

25   microcapsule and macromer solution. Hence, the polymerization and gelation (if multifunctional macromer is used) initially takes place only at the interface, just beneath it, and just beyond it. If left for longer periods of time, the dye starts

- 22 -

5   diffusing from the inner core of the microsphere into the

solution; similarly, macromers start diffusing inside the core.

        Polymerization and subsequent gelation are very rapid

(typical gelation times are 100 ms) (Fouassier, et al., (1985) J.

Polym. Sci., Polym. Chem. Ed., 23:569; Chesneau, et al., (1985)

10   Die Ange. Makromol. Chemie, 135:41, (1988) Makromol. Chem., Rapid

Commun., 9:223). Because diffusion is a much slower process than

polymerization, not the entire macromer solution is polymerized

or gelled. Essentially the reaction is restricted to the near

surface only. The dye, being a smaller molecule and being weakly

15   bound to the capsule materials, keeps diffusing out of the

microsphere. If this diffusion occurs under laser irradiation,

then dye at the interface is used continuously to form a thicker

gel layer. The thickness of the coating can thus be directed by

controlling the reaction conditions.

20   A schematic representation of this process is shown in

Figure 2A. The amount, thickness or size and rigidity of the gel

formed will depend on the size and intensity of the beam, time of

exposure, initiator, macromer molecular weight, and macromer

concentration (see below). Alginate/PLL microspheres containing

25   islets coated by this technique are shown in Figure 2B.

        4.   DIRECT INTERFACIAL POLYMERIZATION METHOD

        The fourth embodiment of this invention utilizes

interfacial polymerization to form a membrane directly on the

surface of the biological material. This results in the smallest

- 23 -

5   capsules and thus achieves optimal economy of volume. Tissue is

directly coated with photoinitiator, emersed in the macromer

solution, and immediately irradiated. This technique results in

a thin polymer coat surrounding the tissue since there is no

space taken up by a microcapsule, and the photoinitiator is again

10   present only in a thin shell of the macromer solution.

USE AS AN ADHESIVE

It is usually difficult to get good adhesion between

polymers of greatly different physicochemical properties. The

concept of a surface physical interpenetrating network was

15   presented by Desai and Hubbel (Desai et al. (1992) Macromolecules

25:226). This approach to incorporating into the surface of one

polymer a complete coating of a polymer of considerably different

properties involved swelling the surface of the polymer to be

modified (base polymer) in a mutual solvent, or a swelling

20   solvent, for the base polymer and for the polymer to be

incorporated (penetrant polymer). The penetrant polymer diffused

into the surface of the base polymer. This interface was

stabilized by rapidly precipitating or deswelling the surface by

placing the base polymer in a nonsolvent bath. This resulted in

25   entanglement of the penetrant polymer within the matrix of the

base polymer at its surface in a structure that was called a

surface physical interpenetrating network.

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5   This approach can be improved upon by photopolymerizing
the penetrant polymer upon the surface of the base polymer in the
swollen state. This results in much enhanced stability over that
of the previous approach and in the enhancement of biological
responses to these materials. The penetrant may be chemically

10   modified to be a prepolymer (macromer), i.e. capable of being
polymerized itself. This polymerization can be initiated
thermally or by exposure to visible, ultraviolet, infrared, gamma
ray, or electron beam irradiation, or to plasma conditions. In
the case of the relatively nonspecific gamma ray or electron beam

15   radiation reaction, chemical incorporation of particularly
reactive sites may not be necessary.

        Polyethylene glycol (PEG) is a particularly useful
penetrant polymer for biomedical applications where the lack of
cell adhesion is desired. The previous work had demonstrated an

20   optimal performance at a molecular weight of 18,500 D without
chemical crosslinking. PEG prepolymers can be readily formed by
acrylation of the hydroxyl groups at its termini or elsewhere
within the chain. These prepolymers can be readily polymerized
by the above described radiation methods. Photoinititated

25   polymerization of these prepolymers is particularly convenient
and rapid. There are a variety of visible light initiated and
ultraviolet light initiated reactions that are initiated by light
absorption by specific photochemically reactive dyes, described
elsewhere herein. This same approach can be used for biomedical

- 25 -

5   purposes with other water-soluble polymers, such as poly(N-vinyl

pyrrolidinone), poly(N-isopropyl acrylamide), poly(ethyl

oxazoline) and many others.

Additionally, it is usually difficult to obtain

adhesives for wet surfaces and tissues. Water soluble

10   prepolymers, for example PEG diacrylates, can be used for this

purpose. When a water soluble polymer is placed in aqueous

solution upon a tissue, the polymer diffuses into the surface of

the tissue, within the protein and polysaccharide matrix upon the

tissue but not within the cells themselves. When the water

15   soluble polymer is a prepolymer and a visible, ultraviolet or

infrared photoinitiator is included, the polymer penetrant may be

exposed to the appropriate light to gel the polymer. In this

way, the polymer is crosslinked within and around the matrix of

the tissue in what is called an interpenetrating network. If the

20   prepolymer is placed in contact with two tissues and the

prepolymer is illuminated, then these two tissues are adhered

together by the intermediate polymer gel.

BIOLOGICAL MATERIALS

Due to the biocompatibility of the materials and

25   techniques involved, a wide variety of materials can be used in

conjunction with the present invention. For encapsulation, the

techniques can be used with mammalian tissue and/or cells, as

well as sub-cellular organelles and other isolated sub-cellular

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5   components.   The membranes can be crafted to meet the perm-

selectivity needs of the biological material enclosed.   Cells

which are to be used to produce desired products such as proteins

are optimally encapsulated by this invention.

Examples of cells which can be encapsulated are primary

10   cultures as well as established cell lines, including transformed

cells.   These include but are not limited to pancreatic islet

cells, human foreskin fibroblasts, Chinese hamster ovary cells,

beta cell insulomas, lymphoblastic leukemia cells, mouse 3T3

fibroblasts, dopamine secreting ventral mesencephalon cells,

15   neuroblastoid cells, adrenal medulla cells, and T-cells.   As can

be seen from this partial list, cells of all types, including

dermal, neural, blood, organ, muscle, glandular, reproductive,

and immune system cells can be encapsulated successfully by this

method.   Additionally, proteins (such as hemoglobin),

20   polysaccharides, oligonucleotides, enzymes (such as adenosine

deaminase), enzyme systems, bacteria, microbes, vitamins,

cofactors, blood clotting factors, drugs (such as TPA,

streptokinase or heparin), antigens for immunization, hormones,

and retroviruses for gene therapy can be encapsulated by these

25   techniques.

The biological material can be first enclosed in a

structure such as a polysaccharide gel.   (Lim, U.S.P.N.

4,352,883; Lim, U.S.P.N. 4,391909; Lim, U.S.P.N. 4,409,331;

Tsang, et al., U.S.P.N. 4,663,286; Goosen et al., U.S.P.N.

5   4,673,556; Goosen et al., U.S.P.N. 4,689,293; Goosen et al.,

U.S.P.N. 4,806,355; Rha et al., U.S.P.N. 4,744,933; Rha et al.,

U.S.P.N. 4,749,620, incorporated herein by reference.) Such gels

can provide additional structural protection to the material, as

well as a secondary level of perm-selectivity. If alginate is

10   ·used, it is preferred that the alginate be relatively high in α-

L-guluronic acid content. This "high G" content increases the

biocompatibility of the material. The alginate should be at

least 60% α-L-guluronic acid, and more preferably at least 70% α-
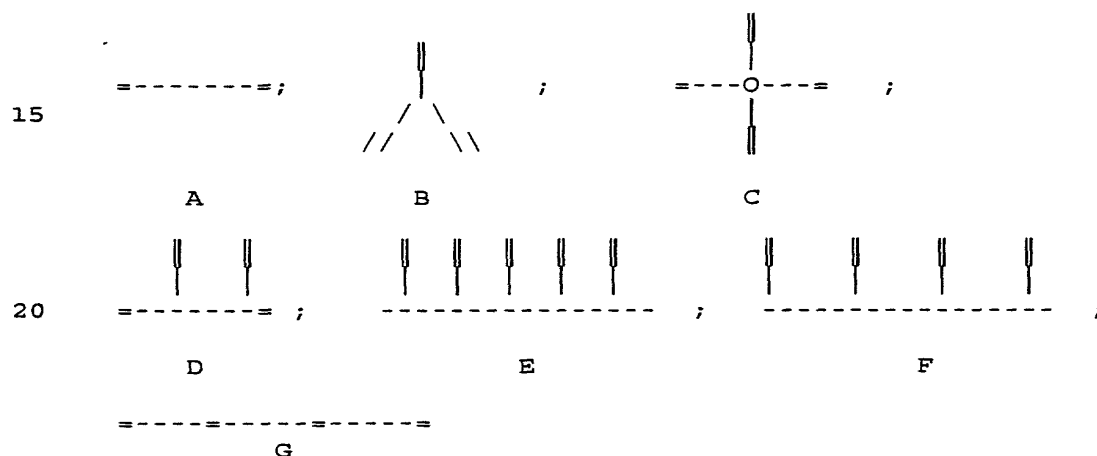
L-guluronic acid.

15   <u>MACROMERS</u>

          Polymerization via this invention utilizes macromers

rather than monomers as the building blocks. The macromers are

small polymers which are susceptible to polymerization into the

larger polymer membranes of this invention. Polymerization is

20   enabled because the macromers contain sites of unsaturation,

e.g., carbon-carbon double bond moieties, carbon-carbon triple

bond moieties, and the like, as well as sites of unsaturation

between carbon atoms and heteroatoms and between two heteroatoms.

Examples of carbon-carbon double bonds useful in this invention

25   include acrylate, methacrylate, ethacrylate, 2-phenyl acrylate,

2-chloro acrylate, 2-bromo acrylate, itaconate, acrylamide,

methacrylamide, and styrene groups. The macromers are water

5   soluble compounds and are non-toxic to biological material before

and after polymerization.

A wide variety of substantially water soluble polymers

exist, some of which are illustrated schematically below.

(_____) represents a substantially water soluble region of the

10   polymer, and (=) represents a free radical polymerizable species.

Examples include:

```
                        ‖                    ‖
                        |                    |
   =-------=;           |        ;       =---O---=    ;
                       / \                   |
15                    // \\                  ‖

     A                  B                    C

     ‖     ‖          ‖  ‖  ‖  ‖  ‖        ‖    ‖    ‖    ‖    ‖
     |     |          |  |  |  |  |        |    |    |    |    |
20 =-------= ;     ----------------- ;   ----------------- ;

     D                  E                        F

   =----=-----=-----=
            G
```

Examples of A include PEG diacrylate, from a PEG diol;

25   of B include PEG triacrylate, formed from a PEG triol; of C

include PEG-cyclodextrin tetraacrylate, formed by grafting PEG to

a cyclodextrin central ring, and further acrylating; of D include

PEG tetraacrylate, formed by grafting two PEG diols to a bis

epoxide and further acrylating; of E include hyaluronic acid

30   methacrylate, formed by acrylating many sites on a hyaluronic

acid chain; of F include PEG-hyaluronic acid-multiacrylate,

- 29 -

5   formed by grafting PEG to hyaluronic acid and further acrylating;

of G include PEG-unsaturated diacid ester formed by esterifying a

PEG diol with an unsaturated diacid.

    Polysaccharides include, for example, alginate

(preferably high-G alginate), hyaluronic acid, chondroitin

10   sulfate, dextran, dextran sulfate, heparin, heparin sulfate,

heparan sulfate, chitosan, gellan gum, xanthan gum, guar gum, and

K-carrageenan.   Proteins, for example, include gelatin, collagen,

elastin and albumin, whether produced from natural or recombinant

sources.   In the event that a substance such as alginate or

15   hyaluronic acid is used, the resulting macromers will be both

ionically crosslinkable and covalently crosslinkable.

    Photopolymerizable substituents preferably include

acrylates, diacrylates, oligoacrylates, dimethacrylates, or

oligomethoacrylates, and other biologically acceptable

20   photopolymerizable groups.

Synthetic Polymeric Macromers

    The water-soluble macromer may be derived from water-

soluble polymers including, but not limited to, poly(ethylene

oxide) (PEO), poly(ethylene glycol) (PEG), poly(vinyl alcohol)

25   (PVA), poly(vinylpyrrolidone) (PVP), poly(ethyloxazoline) (PEOX)

polyaminoacids, pseudopolyamino acids, and polyethyloxazoline, as

well as copolymers of these with each other or other water

soluble polymers or water insoluble polymers, provided that the

conjugate is water soluble.   An example of a water soluble

- 30 -

5   conjugate is a block copolymer of polyethylene glycol and

polypropylene oxide, commercially available as a Pluronic™

surfactant.

Polysaccharide Macromers

Polysaccharides such as alginate (preferably high-G

10   alginate), hyaluronic acid, chondroitin sulfate, dextran, dextran

sulfate, heparin, heparin sulfate, heparan sulfate, chitosan,

gellan gum, xanthan gum, guar gum, water soluble cellulose

derivatives, and carrageenan, which are linked by reaction with

hydroxyls or amines on the polysaccharides can also be used to

15   form the macromer solution. As noted above, in the event that

alginate, hyaluronic acid, or other ionically gellable materials

are used, the macromers are both ionically crosslinkable and

covalently crosslinkable.

Protein Macromers

20   Proteins such as gelatin, collagen, elastin, zein, and

albumin, whether produced from natural or recombinant sources,

which are made free-radical polymerization by the addition of

carbon-carbon double or triple bond-containing moieties,

including acrylate, diacrylate, methacrylate, ethacrylate, 2-

25   phenyl acrylate, 2-chloro acrylate, 2-bromo acrylate, itaconate,

oliogoacrylate, dimethacrylate, oligomethacrylate, acrylamide,

methacrylamide, styrene groups, and other biologically acceptable

photopolymerizable groups, can also be used to form the macromer

solution.

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5   MACROMERS FOR BIODEGRADABLE GELS

            In general terms, the macromers for biodegradable gels

are polymers that are soluble in aqueous solutions, or nearly

aqueous solutions, such as water with added dimethylsulfoxide.

They have three components including a biodegradable region,

10   preferably hydrolyzable under *in vivo* conditions, a water soluble

region, and at least two polymerizable regions. Examples of

these structures are shown in Figure 1.

            Structure A in Figure 1 shows a macromer having a water

soluble region (_____), a water soluble and degradable component

15   (------) appended to one another. Each has a polymerizable end

cap (======). Structure B shows a major water soluble component

or core region (_____) extended at either end by a degradable or

hydrolyzable component (~~~~~) and terminated by, at either end,

a polymerizable component (======). Structure C shows a central

20   degradable or hydrolyzable component (~~~~~) bound to a water

soluble component (_____) capped at either end by a

polymerizable component (======). Structure D shows a central

water soluble component (_____) with numerous branches of

hydrolyzable components (~~~~~), each hydrolyzable component

25   being capped with a polymerizable component (======). Structure

E shows a central biodegradable, hydrolyzable component (~~~~~)

with three water soluble branches (_____), each water soluble

branch being capped by a polymerizable component (======).

Structure F shows a long central water soluble and hydrolyzable

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5   component (------), each end being capped by a polymerizable

component (======). Structure G shows a central water soluble

and hydrolyzable component (------) capped at both ends by a

hydrolyzable component (~~~~~), each hydrolyzable component

being capped by a polymerizable component (======). Structure H

10 shows a central water soluble and degradable or hydrolyzable

component (------) with end caps or branches of a polymerizable

component (======). Structure I shows a central water soluble

component (_____) in circular form with water soluble branches

extended by a hydrolyzable component (~~~~~) capped by a

15 polymerizable component (======). Lastly, Structure J in Figure

1 shows a circular water soluble core component (_____) with

degradable branches (~~~~~), each being capped by a

polymerizable component (~~~~~).

The various structures shown in Figure 1 are exemplary

20 only. Those skilled in the art will understand many other

possible combinations which could be utilized for the purposes of

the present invention.

Used herein is the term "at least substantially water

soluble." This is indicative that the solubility should be at

25 least about 1 g/100 ml of aqueous solution or in aqueous solution

containing small amounts of organic solvent, such as

dimethylsulfoxide. By the term "polymerizable" is meant that the

regions have the capacity to form additional covalent bonds

resulting in macromer interlinking, for example, carbon-carbon

- 33 -

5   double bonds of acrylate-type molecules. Such polymerization is

characteristically initiated by free-radical formation, for

example, resulting from photon absorption of certain dyes and

chemical compounds to ultimately produce free-radicals.

In a preferred embodiment, a hydrogel begins with a

10   biodegradable, polymerizable, macromer including a core, an

extension on each end of the core, and an end cap on each

extension. The core is a hydrophilic polymer or oligomer; each

extension is a biodegradable polymer or oligomer; and each end

cap is an oligomer, dimer or monomer capable of cross-linking the

15   macromers. In a particularly preferred embodiment, the core

includes hydrophilic poly(ethylene glycol) oligomers of molecular

weight between about 400 and 30,000 Da; each extension includes

biodegradable poly (α-hydroxy acid) oligomers of molecular weight

between about 200 and 1200 Da; and each end cap includes an

20   acrylate-type monomer or oligomer (i.e., containing carbon-carbon

double bonds) of molecular weight between about 50 and 200 Da

which are capable of cross-linking and polymerization between

copolymers. More specifically, a preferred embodiment

incorporates a core consisting of poly(ethylene glycol) oligomers

25   of molecular weight between about 8,000 and 10,000 Da; extensions

consisting of poly(lactic acid) oligomers of molecular weight

about 250 Da; and end caps consisting acrylate moieties of about

100 Da molecular weight.

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5   Those skilled in the art will recognize that oligomers

of the core, extensions and end caps may have uniform

compositions or may be combinations of relatively short chains or

individual species which confer specifically desired properties

on the final hydrogel while retaining the specified overall

10   characteristics of each section of the macromer. The lengths of

oligomers referred to herein may vary from two mers to many, the

term being used to distinguish subsections or components of the

macromer from the complete entity.

Water Soluble Regions

15   In preferred embodiments, the core water soluble region

can consist of poly(ethylene glycol), poly(ethylene oxide),

poly(vinyl alcohol), poly(vinylpyrrolidone),

poly(ethyloxazoline), poly(ethylene oxide)-co-

poly(propyleneoxide) block copolymers, polysaccharides or

20   carbohydrates such as hyaluronic acid, dextran, heparan sulfate,

chondroitin sulfate, heparin, or alginate (preferably high-G

alginate), proteins such as gelatin, collagen, albumin,

ovalbumin, or polyamino acids.

Biodegradable Regions

25   The biodegradable region is preferably hydrolyzable

under *in vivo* conditions. For example, hydrolyzable group may be

polymers and oligomers of glycolide, lactide, e-caprolactone,

other hydroxy acids, and other biologically degradable polymers

that yield materials that are non-toxic or present as normal

- 35 -

5   metabolites in the body. Preferred poly(α-hydroxy acid)s are

poly(glycolic acid), poly(DL-lactic acid) and poly(L-lactic

acid). Other useful materials include poly(amino acids),

poly(anhydrides), poly(orthoesters), poly(phosphazines) and

poly(phosphoesters). Polylactones such as poly(ϵ-caprolactone),

10   poly(ϵ-caprolactone), poly(δ-valerolactone) and poly(gamma-

butyrolactone), for example, are also useful. The biodegradable

regions may have a degree of polymerization ranging from one up

to values that would yield a product that was not substantially

water soluble. Thus, monomeric, dimeric, trimeric, oligomeric,

15   and polymeric regions may be used.

Biodegradable regions can be constructed from polymers

or monomers using linkages susceptible to biodegradation, such as

ester, peptide, anhydride, orthoester, phosphazine and

phosphoester bonds.

20   <u>Polymerizable Regions</u>

The polymerizable regions are preferably polymerizable

by photoinitiation by free radical generation, most preferably in

the visible or long wavelength ultraviolet radiation. The

preferred polymerizable regions are acrylates, diacrylates,

25   oligoacrylates, methacrylates, dimethacrylates,

oligomethoacrylates, or other biologically acceptable

photopolymerizable groups.

Other initiation chemistries may be used besides

photoinitiation. These include, for example, water and amine

- 36 -

5   initiation schemes with isocyanate or isothiocyanate containing

macromers used as the polymerizable regions.

MACROMER SIZE

These macromers can vary in molecular weight from 0.2-

100 kD, depending on the use. The degree of polymerization, and

10   the size of the starting macromers, directly affect the porosity

of the resulting membrane. Thus, the size of the macromers are

selected according to the permeability needs of the membrane.

For purposes of encapsulating cells and tissue in a manner which

prevents the passage of antibodies across the membrane but allows

15   passage of nutrients essential for cellular metabolism, the

preferred starting macromer size is in the range of 10 kD to 18.5

kD, with the most preferred being around 18.5 kD. Smaller

macromers result in polymer membranes of a higher density with

smaller pores.

20   PHOTOINITIATING DYES

The photoinitiating dyes capture light energy and

initiate polymerization of the macromers. Any dye can be used

which absorbs light having frequency between 320 nm and 900 nm,

can form free radicals, is at least partially water soluble, and

25   is non-toxic to the biological material at the concentration used

for polymerization. Examples of suitable dyes are ethyl eosin,

eosin Y, fluorescein, 2,2-dimethoxy,2-phenylacetophenone, 2-

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5   methoxy,2-phenylacetophenone, camphorquinone, rose bengal,

methylene blue, erythrosin, phloxime, thionine, riboflavin and

methylene green. The preferred initiator dye is ethyl eosin due

to its spectral properties in aqueous solution.

COCATALYST

10   The cocatalyst is a nitrogen based compound capable of

stimulating the free radical reaction. Primary, secondary,

tertiary or quaternary amines are suitable cocatalysts, as are

any nitrogen atom containing electron-rich molecules.

Cocatalysts include, but are not limited to, triethanolamine,

15   triethylamine, ethanolamine, N-methyl diethanolamine, N,N-

dimethyl benzylamine, dibenzyl amine, N-benzyl ethanolamine, N-

isopropyl benzylamine, tetramethyl ethylenediamine, potassium

persulfate, tetramethyl ethylenediamine, lysine, ornithine,

histidine and arginine.

20   RADIATION WAVELENGTH

The radiation used to initiate the polymerization is

either longwave UV or visible light, with a wavelength in the

range of 320-900 nm. Preferably, light in the range of 350-700

nm, and even more preferred in the range of 365-514 nm, is used.

25   This light can be provided by any appropriate source able to

generate the desired radiation, such as a mercury lamp, longwave

UV lamp, He-Ne laser, or an argon ion laser.

5   THICKNESS AND CONFORMATION OF POLYMER LAYER

               Membrane thickness affects a variety of parameters,
       including perm-selectivity, rigidity, and size of the membrane.
       In the interfacial polymerization method, the duration of the
       radiation can be varied to adjust the thickness of the polymer
10   membrane formed. This correlation between membrane thickness and
       duration of irradiation occurs because the photoinitiator
       diffuses at a steady rate, with diffusion being a continuously
       occurring process. Thus, the longer the duration of irradiation,
       the more photoinitiator will initiate polymerization in the
15   macromer mix, the more macromer will polymerize, and a thicker
       coat will be formed. Additional factors which affect membrane
       thickness are the number of reactive groups per macromer, the
       concentration of accelerators in the macromer solution. This
       technique allows the creation of very thin membranes because the
20   photoinitiator is first present in a very thin layer at the
       surface of the biological material, and polymerization only
       occurs where the photoinitiator is present.

               The suspension polymerization method forms a somewhat
       thicker membrane than the interfacial polymerization method.
25   This is because polymerization occurs in the suspension method
       throughout the macromer mix. The thickness of membranes formed
       by the suspension method is determined in part by the viscosity
       of the macromer solution, the concentration of the macromer in
       that solution, the fluid mechanical environment of the suspension

5 and surface active agents in the suspension. These membranes
vary in thickness from 50-300 microns. The shape of the
structure formed by suspension polymerization can be controlled
by shaping the reaction mix prior to polymerization. Spheres can
be formed by emulsion with a non-miscible liquid such as oil,

10 coextrusion with such a liquid, or coextrusion with air.
Cylinders may be formed by casting or extrusion, and slabs and
discoidal shapes can be formed by casting. Additionally, the
shape may be formed in relationship to an internal supporting
structure such as a screening network of stable polymers (e.g. an

15 alginate gel, preferably high-G alginate, or a woven polymer
fiber) or nontoxic metals.

The overall amount, thickness, and rigidity of the
membrane formed depends on the interaction of several parameters,
including the size and intensity of the radiation beam, duration

20 of exposure of the solution to the radiation, reactivity of the
initiator selected, macromer molecular weight, and macromer
concentration.

APPLICATIONS FOR THE MACROMERS

The invention can be used for a variety of purposes,

25 some of which are enumerated below, along with benefits which
accrue from the use of the invention. Some additional purposes
are illustrated by the Examples which follow.

- 40 -

5 a. Microencapsulating cells: more biocompatible, stronger, more stable, better control of permselectivity, less toxic conditions

   b. Macroencapsulating cells: more biocompatible, stronger, more stable, better control of permselectivity, less

10   toxic conditions, easier to incorporate internal or external supporting structure

   c. Microencapsulating or macroencapsulating other tissues, with the same benefits

   d. Microencapsulating or macroencapsulating pharmaceuti-

15   cals: more biocompatible, less damaging to the pharmaceutical

   e. Coating devices: ease of application, more biocompatible

   f. Coating microcapsules: more biocompatible, strengthens

20   them, ease of coating

   g. Coating macrocapsules, microcapsules, microspheres and macrospheres: more biocompatible, ease of coating

   h. Coating tissues to alter adhesion of other tissues: ease of coating, less toxicity to the tissues,

25   conformal coating versus nonconformal

   i. Adhesive between two tissues: ease of adhesion, rapidity of forming adhesive bond, less toxicity to tissues

5   Applications for the Biodegradable Macromers

Further, the biodegradable macromers can be used for

the following purposes.

Prevention of Surgical Adhesions

A preferred application is a method of reducing

10   formation of adhesions after a surgical procedure in a patient.

The method includes coating damaged tissue surfaces in a patient

with an aqueous solution of a light-sensitive free-radical

polymerization initiator and a macromer solution as described

above.   The coated tissue surfaces are exposed to light

15   sufficient to polymerize the macromer. The light-sensitive free-

radical polymerization initiator may be a single compound (e.g.,

2,2-dimethoxy-2-phenyl acetophenone) or a combination of a dye

and a cocatalyst (e.g., ethyl eosin and triethanol amine).

Controlled drug delivery

20   A second preferred application concerns a method of

locally applying a biologically active substance to tissue

surfaces of a patient. The method includes the steps of mixing a

biologically active substance with an aqueous solution including

a light-sensitive free-radical polymerization initiator and a

25   macromer as described above to form a coating mixture. Tissue

surfaces are coated with the coating mixture and exposed to light

sufficient to polymerize the macromer. The biologically active

substance can be any of a variety of materials, including

proteins, carbohydrates, nucleic acids, and inorganic and organic

5   biologically active molecules. Specific examples include

enzymes, antibiotics, antineoplastic agents, local anesthetics,

hormones, antiangiogenic agents, antibodies, neurotransmitters,

psychoactive drugs, drugs affecting reproductive organs, and

oligonucleotides such as antisense oligonucleotides.

10   In a variation of the method for controlled drug

delivery, the macromers are polymerized with the biologically

active materials to form microspheres or nanoparticles containing

the biologically active material. The macromer, photoinitiator,

and agent to be encapsulated are mixed in an aqueous mixture.

15   Particles of the mixture are formed using standard techniques,

for example, by mixing in oil to form an emulsion, forming

droplets in oil using a nozzle, or forming droplets in air using

a nozzle. The suspension or droplets are irradiated with a light

suitable for photopolymerization of the macromer.

20   **Tissue Adhesives**

Another use of the polymers is in a method for adhering

tissue surfaces in a patient. The macromer is mixed with a

photoinitiator or photoinitiator/cocatalyst mixture to form an

aqueous mixture and the mixture is applied to a tissue surface to

25   which tissue adhesion is desired. The tissue surface is

contacted with the tissue with which adhesion is desired, forming

a tissue junction. The tissue junction is then irradiated until·

the macromers are polymerized.

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5   ## Tissue Coatings

In a particularly preferred application of these macromers, an ultrathin coating is applied to the surface of a tissue, most preferably the lumen of a tissue such as a blood vessel. One use of such a coating is in the treatment or

10   prevention of restenosis, abrupt reclosure, or vasospasm after vascular intervention. The photoinitiator is applied to the surface of the tissue, allowed to react, adsorb or bond to tissue, the unbound photoinitiator is removed by dilution or rinsing, and the macromer solution is applied and polymerized.

15   As demonstrated below, this method is capable of creating uniform polymeric coating of between one and 500 microns in thickness, most preferably about twenty microns, which does not evoke thrombosis or localized inflammation.

Tissue Supports

20   The macromers can also be used to create tissue supports by forming shaped articles within the body to serve a mechanical function. Such supports include, for example, sealants for bleeding organs, sealants for bone defects and space-fillers for vascular aneurisms. Further, such supports

25   include strictures to hold organs, vessels or tubes in a particular position for a controlled period of time.

The invention described herein is further exemplified in the following Examples. While these Examples provide a

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5   variety of combinations useful in performing the methods of the

invention, they are illustrative only and are not to be viewed as

limiting in any manner the scope of the invention.


Example 1

Synthesis of PEG 6 kD Diacrylate

10   PEG acrylates of molecular weights 400 Da and 1,000 Da

ware commercially available from Sartomer and Dajac Inc.,

respectively.   PEG 6 kD (20 g) was dissolved in 200 mL

dichloromethane in a 250 mL round bottom flask.   The flask was

cooled to 0°C and 1.44 mL of triethyl amine and 1.3 mL of

15   acryloyl chloride were added with constant stirring under a dry

nitrogen atmosphere.   The reaction mixture was then brought to

room temperature and stirred for 12 hr under a nitrogen

atmosphere.   It was then filtered, and the filtrate was

precipitated by adding to a large excess of hexane.   The crude

20   monomer was purified by dissolving in dichloromethane and

precipitating in hexane.   Yield 69%.

Example 2

Synthesis of PEG 18.4 kD Tetraacrylate

A tetrafunctional water soluble PEG (30 g; m.w. 18.5

25   kD) having the following structure was purchased from

Polysciences, Inc.:

$$HO-(CH_2-CH_2-O)_n-CH_2-CH-CH_2- \quad -\overset{\overset{\displaystyle CH_3}{|}}{C}- \quad -CH_2-CH-CH_2-(O-CH_2-CH_2)_n-OH$$
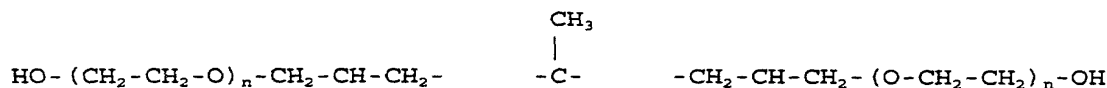
5   where F_1 = CONH, COO or NHCOO

       X = H, CH_3, C_2H_5, C_6H_5, Cl, Br, OH or CH_2COOH

       F_2 = COO, CONH, O or C_6H_4, AND

       R = CH_2 or -alkyl-.

       The PEG was dried by dissolving in benzene and

10   distilling off the water-benzene azeotrope.   PEG 18.5 kD (59 g)

was dissolved in 300 mL of benzene in a 500 mL flask.   To this,

3.6 mL of triethylamine and 2.2 mL of acryloyl chloride were

added under nitrogen atmosphere and the reaction mixture was

refluxed for 2 hours.   It was then cooled and stirred overnight.

15   The triethyl amine hydrochloride was separated by filtration and

the copolymer was recovered from filtrate by precipitating in a

large excess of hexane.   The polymer was further purified by

dissolving in methylene chloride and reprecipitating in hexane.

The polymer was dried at 50°C under vacuum for 1 day.   Yield 68%.

20   **Example 3**

           Coating of Islet-Containing Alginate-PLL

           Microspheres by Surface Dye Adsorption

       The microcapsule interfacial polymerization method was

used to form membrane around alginate-PLL microcapsules

                          - 46 -

5   containing islets. Alginate-PLL coacervated microspheres,

containing one or two human pancreatic islets each, were

suspended in a 1.1% $CaCl_2$ solution and aspirated free of excess

solution to obtain a dense plug of microspheres. A solution of

ethyl eosin (0.04% w/v) was prepared in a 1.1 % $CaCl_2$ solution.

10   This solution was filter-sterilized by passage through a 0.45μm

filter. The plug of microspheres was suspended in 10 mL of the

eosin solution for 2 min to allow uptake of the dye. The

microspheres were then washed four times with fresh 1.1% $CaCl_2$ to

remove excess dye. A solution of PEG 18.5 tetraacrylate (2 mL;

15   23% w/v) containing 100μL of a 3.5% w/v solution of

triethanolamine in HEPES buffered saline was added to 0.5 mL of

these microspheres. The microspheres were exposed to argon ion

laser light for 30 seconds with periodic agitation. The

suspension of microspheres was uniformly scanned with the light

20   during this period. The microspheres were then washed with

calcium solution and the process was repeated in order to further

stabilize the coating.

      A static glucose stimulation test (SGS) was performed

on islets encapsulated in the microspheres coated with PEG gel.

25   Data for insulin secretion in response to this challenge appears

in Table 1. The islets were seen to be viable by dithizone

staining. The SGS test data confirm the vitality and

functionality of the islets.

5

TABLE 1

SGS

	initial	pulse	subsequent
Glucose Concentration (mg%)	60	300	60
	Insulin/Islet/hr	(μU/mL) *	
10 Diffusion Overcoat Method	1.0	10.04±3.56	2.5450.76
Mineral Oil Overcoat Method	1.0	10.23±3.28	1.0250.78
Free Islet Control	1.0	3.74±1.4	1.950.17

* Values are mean ± S.D., all are normalized as compared to the initial 60 mg %, after subjection to the 300 mg % glucose, the

15 islets were resubjected to the initial dose.

        PEG diacrylate macromers may be polymerized identically as the PEG tetraacrylate macromer described in this example.

Example 4

Coating Islet-Containing Alginate-PLL Microspheres

20 ### by the Microcapsule Suspension Polymerization Method

        This method takes advantage of the hydrophilic nature of PEG monomers. Alginate/PLL microspheres (2 mL), containing one or two human pancreatic islets each, were mixed with PEG tetraacrylate macromer solution (PEG mol wt 18.5 kD, 23% solution

25 in saline) in a 50 mL transparent centrifuge tube. Triethanolamine (0.1M) and 0.5 mM ethyl eosin were mixed with macromer solution. The excess of macromer solution was decanted,

- 48 -

5   20 mL of mineral oil was added to the tube, and the reaction

mixture was vortexed thoroughly for 5 minutes. Silicone oil will

perform equally well in this synthesis but may have poorer

adjuvant characteristics if there is any carry-over. Any other

water-immiscible liquid may be used as the "oil" phase.

10   Acceptable triethanolamine concentrations range from about 1 mM

to about 100mM. Acceptable ethyl eosin concentrations range from

about 0.01 mM to more than 10 mM.

The beads were slightly red due to the thin coating of

macromer/dye solution, and they were irradiated for 20-50 sec

15   with an argon ion laser (power 50-500 mW). Bleaching of the

(red) ethyl eosin color suggested completion of the reaction.

The beads were then separated from mineral oil and washed several

times with saline solution. The entire procedure was carried out

under sterile conditions.

20   A schematic representation of the microsphere coating

process in oil is shown in Figure 3. Alginate/polylysine

capsules are soluble in sodium citrate at pH 12. When these

coated microspheres came in contact with sodium citrate at pH 12,

the inner alginate/polylysine coacervate dissolves and a PEG

25   polymeric membrane could still be seen (crosslinked PEG gels are

substantially insoluble in all solvents including water and

sodium citrate at pH 12). The uncoated control microspheres

dissolved completely and rapidly in the same solution.

5   A static glucose challenge was performed on the islets

as in Example 3. Data again appear in Table 1. The islets were

seen to be viable and functional.

Example 5

Encapsulation of Islets of Langerhans

10   This example makes use of the direct interfacial

polymerization. Islets of Langerhans isolated from a human

pancreas were encapsulated in PEG tetraacrylate macromer gels.

500 islets suspended in RPMI 1640 medium containing 10% fetal

bovine serum were pelleted by centrifuging at 100g for 3 min.

15 The pellet was resuspended in 1 mL of a 23% w/v solution of PEO

18.5 kD diacrylate macromer in HEPES buffered saline. An ethyl

eosin solution (5 μL) in vinyl pyrrolidone (at a concentration of

0.5%) was added to this solution along with 100 μL of a 5 M

solution of triethanolamine in saline. Mineral oil (20 mL) was

20   then added to the tube which was vigorously agitated to form a

dispersion of droplets 200-500 μm in size. This dispersion was

then exposed to an argon ion laser with a power of 250 mW,

emitting at 514 nm, for 30 sec. The mineral oil was then

separated by allowing the microspheres to settle, and the

25   resulting microspheres were washed twice with PBS, once with

hexane and finally thrice with media.

Figure 4 shows islets of Langerhans encapsulated in a

PEO gel. The viability of the islets was verified by an acridine

- 50 -

5   orange and propidium iodide staining method and also by dithizone

staining. In order to test functional normalcy, an SGS test was

performed on these islets. The response of the encapsulated

islets was compared to that of free islets maintained in culture

for the same time period. All islets were maintained in culture

10   for a week before the SGS was performed. The results are

summarized in Table 2. It can be seen that the encapsulated

islets secreted significantly (p<0.05) higher insulin than the

free islets. The PEO gel encapsulation process did not impair

function of the islets and in fact helped them maintain their

15   function in culture better than if they had not been

encapsulated.

TABLE 2

Islet Insulin secretion

Glucose Concentration (ma%)	60	300	60
20		Insulin/Islet/hr (μU/mL) *	
Free islets	1.0	3.74+/-1.40	1.9+/-0.17
Encapsulated Islets	1.0	20.81+/-9.36	2.0+/-0.76

*Values are mean +/- S.D., normalized to initial basal level at

25   60 mg% glucose.

5   **Example 6**

                    Microencapsulation of Animal Cells

              PEG diacrylates of different molecular weight were

        synthesized by a reaction of acryloyl chloride with PEG as in

        Example 1.   A 20 to 30% solution of macromer was mixed with a

10   cell suspension and the ethyl eosin and triethanolamine

        initiating system before exposing it to laser light through a

        coextrusion air flow apparatus, Figure 5.   Microspheres were

        prepared by an air atomization process in which a stream of

        macromer was atomized by an annular stream of air.   The air flow

15   rate used was 1,600 cc/min and macromer flow-rate was 0.5 mL/min.

        The droplets were allowed to fall to a petri dish containing

        mineral oil and were exposed to laser light for about 0.15 sec

        each to cause polymerization and make them insoluble in water.

        Microspheres so formed were separated from the oil and thoroughly

20   washed with PBS buffer to remove unreacted macromer and residual

        initiator.   The size and shape of microspheres was dependent on

        extrusion rate (0.05 to 0.1 mL/min) and extruding capillary

        diameter (18 Ga to 25 Ga).   The polymerization times were

        dependent on initiator concentration (ethyl eosin concentration

25   (5μM to 0.5mM), vinyl pyrrolidone concentration (0.0% to 0.1 %),

        triethanolamine concentration (5 to 100 mM), laser power (10mW to

        1W), and macromer concentration (>10% w/v).

              A PEG diacrylate macromer of molecular weight 400 Da

        was used as a 30% solution in PBS, containing 0.1M

                                  - 52 -

5   triethanolamine as a cocatalyst and 0.5mM ethyl eosin as a

photoinitiator.   Spheres prepared using this method are shown in

Figure 6.   The polymerizations were carried out at physiological

pH in the presence of air.   This is significant since radical

polymerizations may be affected by the presence of oxygen, and

10   the acrylate polymerization is still rapid enough to proceed

effectively.

The process also works at lower temperatures.   For

cellular encapsulation, a 23% solution of PEO diacrylate was used

with initiating and polymerization conditions as used in the air

15   atomization technique.   Cell viability subsequent to encapsula-

tion was checked by trypan blue exclusion assay.   Human foreskin

fibroblasts (HFF), Chinese hamster ovary cells (CHO-Kl), and a

beta cell insuloma line (RiN5F) were found to be viable (more

than 95%) after encapsulation.   A wide range (> 10%) of PEG

20   diacrylate concentrations may be used equally effectively, as may

PEG tetraacrylate macromers.

Example 7

Coating of Animal Cell-Containing Alginate-PLL
Microspheres and Individual Cells by Surface Dye Adsorption

25   Alginate-PLL coacervated microspheres, containing

animal cells, were suspended in a 1.1% $CaCl_2$ solution and were

aspirated free of excess solution to obtain a dense plug of

microspheres.   A solution was filter sterilized by passage

5   through a 0.45 pm filter. The plug of microspheres was suspended

in 10 mL of eosin solution for 2 min to allow dye uptake. A

solution of PEG 18.5 tetraacrylate (2 mL; 23% w/v) containing 100

μL of a 3.5 w/v solution of triethanolamine in HEPES buffered

saline was added to 0.5 mL of these microspheres. The

10   microspheres were exposed to an argon ion laser for 30 seconds

with periodic agitation. The suspension of microspheres was

uniformly scanned with the laser during this period. The

microspheres were then washed with calcium solution and the

process was repeated once more in order to attain a stable

15   coating.

In order to verify survival of cells after the overcoat

process, cells in suspension without the alginate/PLL micro-

capsule were exposed to similar polymerization conditions. 1 mL

of lymphoblastic leukemia cells (RAJI) ($5X10^5$ cells) was centri-

20   fuged at 300 g for 3 min. A 0.04% filter sterilized ethyl eosin

solution in phosphate buffered saline (PBS) (1 mL) was added and

the pellet was resuspended. The cells were exposed to the dye

for 1 min and washed twice with PBS and then pelleted.

Triethanolamine solution (10 μL; 0.1M) was added to the pellet

25   and the tube was vortexed to resuspend the cells. 0.5 mL of PEO

18.5 kD tetraacrylate macromer was then mixed along with this

suspension and the resulting mixture was exposed to an argon ion

laser (514 nm, 50 mW) for 45 sec. The cells were then washed

twice with 10 mL saline and once with media (RPMI 1640 with 10%

- 54 -

5   FCS and 1% antibiotic, antimycotic). A thin membrane of PEO gel

may be observed forming around each individual cell.

No significant difference in viability was seen between

the control population (93% viable) and the treated cells (95%

viable) by trypan blue exclusion. An assay for cell viability

10   and function was performed by adapting the MTT-Formazan assay for

the RAJI cells. This assay indicates > 90% survival. Similar

assays were performed with two other model cell lines. Chinese

hamster ovary cells (CHO-K1) show no significant difference

$(p<0.05)$ in metabolic function as evaluated by the MTT-Formazan

15   assay. 3T3 mouse fibroblasts also show no significant reduction

$(p<0.05)$ in metabolic activity.

Example 8

Coating Animal Cell Containing Alginate-PLL

Microspheres by the Oil Suspension Method

20   Using the method described in Example 4, RAJI cells

contained in alginate-PLL microspheres were coated with a PEG

polymeric membrane. Viability of these cells was checked by

trypan blue exclusion and they were found to be more than 95%

viable.

Example 9

Coating of Individual Islets of

Langerhans by Surface Dye Adsorption

Using the method described in Example 7, ethyl eosin was adsorbed to the surfaces of islets, exposed to a solution of the PEG macromer with triethanolamine, and exposed to light from an argon-ion laser to form a thin PEG polymeric membrane on the surface of the islets. Islet viability was demonstrated by lack of staining with propidium iodide.

Example 10

Biocompatibility of PEO on Microspheres

In vivo evaluation of the extent of inflammatory response to microspheres prepared in Examples 7 and 8 was carried out by implantation in the peritoneal cavity of mice. Approximately 0.5 mL of microspheres were suspended in 5 mL of sterile HEPES buffered saline. A portion of this suspension (2.5 mL) was injected into the peritoneal cavity of ICR male Swiss white mice. The microspheres were recovered after 4 days by conducting a lavage of the peritoneal cavity with 5 mL of 10U heparin/mL PBS. The extent of cellular growth on the microspheres was visually inspected under a phase contrast microscope. The number of unattached cells present in the recovered lavage fluid was counted using a Coulter counter. Figure 7A shows a photograph of alginate-poly(L-lysine)

5   microspheres explanted after 4 days, while Figure 7B shows

similar spheres which had been coated with PEG gel by the dye

diffusion precess before implantation. As expected, bilayer

alginate-polylysine capsules not containing an outer alginate

layer to provide an extreme test of the ability of the PEG gel

10   layer to enhance the biocompatibility of the bilayer membrane,

were completely covered with cells due to the highly cell

adhesive nature of the PLL surface, whereas the PEG coated

microspheres were virtually free of adherent cells. Almost

complete coverage of alginate-poly(L-lysine) was expected because

15   polylysine has amino groups on the surface, and positively

charged surface amines can interact with cell surface

proteoglycans and support cell growth (Reuveny, et al., (1983)

Biotechnol. Bioeng., 25:469-480). The photographs in Figure 7B.

strongly indicate that the highly charged and cell adhesive

20   surface of PLL is covered by a stable layer of PEG gel. The

integrity of the gel did not appear to be compromised.

The non-cell-adhesive tendency of these microspheres

was evaluated as a percentage of the total microsphere area which

appears covered with cellular overgrowth. These results are

25   summarized in Table 3.

5

TABLE 3

Microsphere Coverage with Cell Overgrowth

Composition of PEG gel	% Cell coverage
18.5 kD	<1
18.5 kD 90%:0.4 kD 10%	<1
18.5 kD 50%:0.4 kD 50%	<1
35k 90%:0.4 kD 10%	5-7
35k 50%: 0.4 kD 50%	<1
Alginate poly(L-lysine)	60-80

An increase in cell count was a result of activation of resident macrophages which secrete chemical factors such as interleukins and induce nonresident macrophages to migrate to the implant site. The factors also attract fibroblasts responsible for collagen synthesis. The variation of cell counts with chemical composition of the overcoat is shown Figure 8 (A-F). It can be seen from the figure that all PEG coated spheres have substantially reduced cell counts. This is consistent with the PEG overcoat generally causing no irritation of the peritoneal cavity.

However, PEG composition does make a difference in biocompatibility, and increasing molecular weights were associated with a reduction in cell counts. This could be due to the gels made from higher molecular weight oligomers having higher potential for steric repulsion due to the longer chain lengths.

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5   **Example 11**

                    Permeability of PEO Gels

          Bovine serum albumin, human IgG, or human fibrinogen

    (20 mg) was dissolved in 2 mL of a 23% w/v solution of oligomeric

    PEO 18.5 kD tetraacrylate in PBS. This solution was laser

10   polymerized to produce a gel 2cm X 2cm X 0.5 cm in size. The

    diffusion of bovine serum albumin, human IgG and human fibrinogen

    (mol wt 66 kD, 150 kD and 350 kD respectively) was monitored

    through the 2cm X 2cm face of these gels using a total protein

    assay reagent (Biorad). A typical release profile for a PEO 18.5

15   kD gel is shown in Figure 9. This gel allowed a slow transport

    of albumin but did not allow IgG and fibrinogen to diffuse. This

    indicates that these gels are capable of being used as

    immunoprotective barriers. This is a vital requirement for a

    successful animal tissue microencapsulation material.

20   The release profile was found to be a function of

    crosslink density and molecular weight of the polyethylene glycol

    segment of the monomer. Figure 10 shows the release of BSA

    through gels made from 23% solutions of PEO diacrylates and

    tetraacrylates of 0.4 kD and 18.5 kD, respectively. It is

25   evident that the 18.5 kD gel is freely permeable to albumin while

    the 0.4 kD gel restricted the diffusion of albumin. The release

    of any substance from these gels will depend on the crosslink

    density of the network and will also depend on the motility of

                            - 59 -

5 the PEG segments in the network. This effect is also dependent

upon the functionality of the macromer. For example, the

permeability of a PEG 18.5 kD tetraacrylate gel is less than that

of an otherwise similar PEG 20 kD diacrylate gel.

In the case of short PEO chains between crosslinks, the

10 "pore" produced in the network will have relatively rigid

boundaries and will be relatively small and so a macromolecule

attempting to diffuse through this gel will be predominantly

restricted by a sieving effect. If the chain length between

crosslinks is long, the chain can fold and move around with a

15 high motility and, besides the sieving effect, a diffusing

macromolecule will also encounter a free volume exclusion effect.

Due to these two contrasting effects a straightforward

relation between molecular weight cutoff for diffusion and the

molecular weight of the starting oligomer is not completely

20 definable. Yet, a desired release profile for a particular

protein or a drug such as a peptide may be accomplished by

adjusting the crosslink density and length of PEG segments.

Correspondingly, a desired protein permeability profile may be

arranged to permit the diffusion of nutrients, oxygen, carbon

25 dioxide, waste products, hormones, growth factors, transport

proteins, and released cellularly synthesized proteins, while

restricting the diffusion of antibodies and complement proteins

and also the ingress of cells, to provide immunoprotectivity to

transplanted cells or tissue. The three dimensional crosslinked

- 60 -

5   covalently bonded polymeric network is chemically stable for

       long-term in vivo applications.

Example 12

Treatment of Silicone Rubber to Enhance Biocompatibility

              Pieces of medical grade silicone rubber (2 X 2 cm) were

10   soaked for 1 h in benzene containing 23% 0.4 kD PEG diacrylate

       and 0.5% 2,2-dimethoxy-2-phenyl acetophenone. The thus swollen

       rubber was irradiated for 15 min with a long wave UV lamp (365

       nm). After irradiation, the sample was rinsed in benzene and

       dried. The air contact angles of silicone rubber under water

15   were measured before and after treatment. The decreased contact

       angle of 500 after treatment, over the initial contact angle of

       630 for untreated silicone rubber reflects an increased

       hydrophilicity due to the presence of the PEG gel on the rubber

       surface.

20   This technique demonstrates that macromer

       polymerization can be used to modify a polymer surface so as to

       enhance biocompatability. For instance, a polyurethane catheter

       can be treated by this method to obtain an implantable device

       coated with PEG. The PEG was firmly anchored to the surface of

25   the polyurethane catheter because the macromer was allowed to

       penetrate the catheter surface (to a depth of 1-2 microns) during

       the soaking period before photopolymerization. Upon irradiation,

5   an interpenetrating network of PEG and polyurethane results. The

PEG was thereby inextricably intertwined with the polyurethane.

Example 13

Treatment of Polyurethane

INTRACATH (Becton Dickinson) polyurethane intravenous

10   catheters (19 ga) were modified at their outer surfaces with

polyethylene glycol diacrylate (PEG DA) of molecular weight 400

and 10000. The prepolymer was dissolved in tetrahydrofuran

(THF), a solvent for the polyurethane, at 50°C, where

polyurethane dissolution is relatively slow. The following

15   solution was prepared and warmed to 50°C:

PEG DA (MW 400)   15%

PEG DA (MW 10000)   15%

THF   70%

with 2,2-dimethoxy, 2-phenyl actophenone at 1.6% of the above

20   solution.

2.5" length catheter segments were closed at one end by

melting a 2 mm length by pressing with a hot metal spatula to

from a flat tab. This tab was used to fix the catheter in the

vessel wall in subsequent animal experiments. The catheter was

25   held with forceps at the tab end and dipped in the treatment

solution for 1-3 sec, pulled out, and the excess fluid shaken

off. The treated catheter was illuminated with an ultraviolet

light (Black Ray, 360 nm) for 2-3 min, rotating the catheter. An

5   untreated control was similarly treated in 70% THF with 30% water

replacing the PEG in the treatment solution.

Following this treatment, both the treated and control

catheters were transferred to 100% methylene chloride to extract

unreacted materials; this extraction was carried out for 36 hr

10   with solvent replacement every 6 hr. These catheters were then

dried and transferred to 70% ethanol, and then into water before

use.

A second composition was also investigated:

PEG DA (MW 400)   10%

15   PEG DA (MW 10000)   15%

Polyethylene oxide (MW 100,000)   5%

THF   70%

with 2,2-dimethoxy, 2-phenyl acetophenone at 1.6% of the above

solution.

20   In this case, the polyethylene oxide of mw 100,000 was

not a prepolymer and was immobilized within the PEG DA matrix by

entanglement, rather than by chemical attachment.

Adult New Zealand male rabbits (7-10 lb) were

anesthetized with rompun-acepromazien-ketamine. The animal was

25   shaved on the ventrolateral jugular and the vessel was raised. A

catheter was inserted into the vessel with the tab outside, and

tied in place via the tab with 4.0 nylon to the adventitia. The

catheter was inserted 1.5 to 2.0" into the vessel. The skin

incision was closed.

5   After a period of 3 days, the animals were euthanized

by overdose of pentobarbital intraperitoneally. The vessel was

again raised and flushed with phosphate buffered saline (PBS) to

superficially rinse away blood between the catheter and the

vessel wall. Two 500 ml bottles, one filled with PBS and one

10   with formalin in PBS were hung from an i.v. pole scaffold, and

the hydraulic differential was used to perfusion fix the vessel.

The vessels were removed proximal and distal to the ends of the

catheters.

The treated catheters were completely wettable, and

15   were very slippery.

A total of 12 rabbits were catheterized for 72 hr. Six

were control, unmodified catheters. These catheters could not be

removed from the vessel wall without dissection, i.e. they were

tightly incorporated into the vessel. These catheters upon

20   removal were red, and the vessel was barely patent. By contrast,

the treated catheters were easily removable, the vessels were

clearly patent, and the catheters were not red. Under the light

microscope, a small amount of white thrombus could be seen on

both formulations of the catheter coating, with somewhat lesser

25   amounts on the formulation containing the polyethylene oxide

100,000.

5   **Example 14**

              Treatment of Ultrafiltration Membranes

          The processes of Examples described above can be

      applied to the treatment of macrocapsular surfaces, such as those

      used for ultrafiltration, hemodialysis and non-microencapsulated

10   immunoisolation of animal tissue. The macrocapsule in this case

      will usually be microporous with a molecular weight cutoff below

      70,000 Da. It may be in the form of a hollow fiber, a spiral

      module, a flat sheet or other configuration. The surface of such

      a macrocapsule can easily be modified using the PEO gel coating

15   process to produce a non-fouling, non-thrombogenic, and non-cell-

      adhesive surface. The coating serves to enhance biocompatibility

      and to offer additional immunoprotection. Materials which can be

      modified in this manner include polysulfones, cellulosic

      membranes, polycarbonates, polyamides, polyimides, polybenzimida-

20   zoles, nylons, and poly(acrylonitrile-co-vinyl chloride)

      copolymers and the like.

          Depending on the physical and chemical nature of the

      surface a variety of methods can be employed to form

      biocompatible overcoats. Hydrophilic surfaces can simply be

25   coated by applying a thin layer of a 30% w/v polymerizable

      solution of PEG diacrylate containing appropriate amounts of dye

      and amine. Hydrophobic surfaces can be first rendered

      hydrophilic by gas plasma discharge treatment and the resulting

      surface can then be similarly coated, or they may simply be

                              - 65 -

5   treated with a surfactant before or during treatment with the PEG

diacrylate solution.

Example 15

Treatment of Textured Materials and Hydrogels

The surface of materials having a certain degree of

10   surface texture, such as woven dacron, dacron velour, and

expanded poly(tetrafluoroethylene) (ePTFE) membranes, was treated

using the coating method described herein. Textured and

macroporous surfaces allow greater adhesion of the PEG gel to the

material surface. This allows the coating of relatively

15.   hydrophobic materials such as PTFE and poly(ethylene

terepthalate) (PET).

Implantable materials such as enzymatic and ion

sensitive electrodes, having a hydrogel (such as poly (HEMA),

crosslinked poly(vinyl alcohol) and poly(vinyl pyrrolidone)) on

20   their surface, are coated with the more biocompatible PEO gel in

a manner similar to the dye adsorption and polymerization

technique used for the alginate-PLL microspheres.

Example 16

Treatment of Dense Materials

25   The surfaces of dense (e.g., nontextured, nongel)

materials such as polymers (including PET, PTFE, polycarbonates,

polyamides, polysulfones, polyurethanes, polyethylene,

5   polypropylene, polystyrene), glass, and ceramics can be treated
       with PEO gel coatings. Hydrophobic surfaces were initially
       treated by a gas plasma discharge to render the surface
       hydrophilic. This ensures better adhesion of the PEO gel coating
       to the surface. Alternatively, coupling agents may be used to
10   increase adhesion, as readily apparent to those skilled in the
       art of polymer synthesis.

                              Example 17

                         Rate of Polymerization

              To demonstrate rapidity of gelation in laser-initiated
15   polymerizations of multifunctional acrylic monomers, the kinetics
       of a typical reaction were investigated. Trimethylolpropyl tri-
       acrylate containing 5×10^{-4} M ethyl eosin as a photoinitiator in
       10 μmoles of N-vinyl pyrrolidone per mL of macromer mix and 0.1M
       of triethanolamine as a cocatalyst, was irradiated with a 500 mW
20   argon ion laser (514 nm wavelength, power 3.05×10^5 W/m^2, beam
       diameter 1 mm, average gel diameter produced 1 mm). A plot of
       the length of the spike of gel formed by penetration of the laser
       beam into the gel versus laser irradiation time is shown in
       Figure 11A. The spikes formed as a result of laser light
25   penetration into the macromer can be seen in Figure 11B.

              A 23% w/w solution of various macromers in HEPES
       buffered saline containing 3 μL of initiator solution (300 mg/mL
       of 2,2-dimethoxy-2-phenylacetophenone in N-vinyl pyrrolidone) was

                              - 67 -

5   used. 100 μL of the solution was placed on a glass coverslip and

irradiated with a low intensity long wave UV (LWUV) lamp

(BlakRay, model 3-100A with flood). The times required for

gelation to occur were noted and are given in Table 4. These

times were typically in the range of 10 seconds.

10   **TABLE 4**

Gelling Time

Polymer Code	Gel Time (sec) (mean ± S.D.)
0.4 kD	6.9 ± 0.5
1 kD	21.3 ± 2.4
6 kD	14.2 ± 0.5
10 kD	8.3 ± 0.2
18.5 kD	6.9 ± 0.1
20 kD	9.0 ± 0.4

20   Time periods of about 10-100 ms were sufficient to gel

a 300 μm diameter droplet (a typical size of gel used in

microencapsulation technology). This rapid gelation, if used in

conjunction with proper choice of macromers, can lead to

entrapment of living cells in a three dimensional covalently

25 bonded polymeric network. The monochromatic laser light will not

be absorbed by the cells unless a proper chromophore is present,

and is considered to be harmless if wavelength is more than about

400 nm. Exposure to long wavelength ultraviolet light (>360 nm)

is harmless at practical intensities and durations.

Example 18

PEO Gel Interactions

Biocompatibility with HFF (human foreskin fibroblasts) cells was demonstrated as follows.

HFF cells were seeded on PEO 18.5 kD tetraacrylate gels at a density of 18,000 cells/cm^2 in Dulbecco's modification of Eagle's medium containing 10% fetal calf serum. The gels were then incubated at 37°C in a 5% CO_2 environment for 4 hr. At the end of this time the gels were washed with PBS to remove any non-adherent cells and were observed under a phase contrast microscope at a magnification of 200X. Figure 12A shows the growth of these cells on a typical PEG gel as compared to glass surface (Figure 12B). The number of attached cells/cm^2 was found to be 510 ± 170 on the gel surfaces as compared to 13,200 ± 3,910 for a control glass surface. The cells on these gels appeared rounded and were not in their normal spread morphology, strongly indicating that these gels do not encourage cell attachment.

Biocompatibility on microspheres was demonstrated as follows. Figure 13 shows a photograph of microspheres explanted from mice as in Example 10; after 4 days very little fibrous overgrowth was seen. The resistance of PEG chains to protein adsorption and hence cellular growth was well documented. Table 5 summarizes the extent of cellular overgrowth seen on these microspheres after 4 day intraperitoneal implants for various PEG diacrylate gels.

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5

TABLE 5

PEG Diacrylate for Gels (mol wt, Daltons)	Extent of Cellular Overgrowth
400	5-10%
1,000	15-25%
5,000	3-5%
6,000	2-15%
10,000	10-20%
18,500	4-10%

10

15

Example 19

Characterization and Mechanical Analysis of PEO Gels

Solutions of PEO diacrylates (23% w/v; 0.4 kD, 6 kD, 10
kD) and PEG tetraacrylates (18.5 kD) were used. An initiator

20   solution (10 μL) containing 30 mg/mL of 2,2-dimethoxy-2-phenyl
acetophenone in vinyl-2-pyrrolidone was used per mL of the
macromer solution. The solution of initiator containing macromer
was placed in a 4.0 X 1.0 X 0.5 cm mold and exposed to a long
wave ultraviolet lamp (365 nm) for approximately 10 seconds to

25   induce gelation. Samples were allowed to equilibrate in
phosphate buffered saline (pH 7.4) for 1 week before analysis 1
performed.

5   A series of "dogbone" samples (samples cut from a slab
into the shape of a dogbone, with wide regions at both ends and a
narrower long region in the middle) were cut for ultimate tensile
strength tests. Thickness of the samples was defined by the
thickness of the sample from which they were cut. These thick-
10   nesses ranged from approximately 0.5 mm to 1.75 mm. The samples
were 20 mm long and 2 mm wide at a narrow "neck" region. The
stress strain tests were run in length control at a rate of 4%
per second. After each test, the cross sectional area was
determined. Table 6 shows the ultimate tensile strength data.
15   It is seen that the lower molecular weight macromers in general
give stronger gels which were less extensible than those made
using the higher molecular weight macromers. The PEG 18.5 kD
tetraacrylate gel is seen to be anomalous in this series,
resulting from the multifunctionality of the macromer and the
20   corresponding higher crosslinking density in the resulting gel.
This type of strengthening result could be similarly achieved
with macromers obtained having other than four free radical
sensitive groups, such as acrylate groups.

5

TABLE 6

Gel strength Tests

| | PEO Acrylate Precursor Molecular Weight | | | |
	0.4 kD	6 kD	10 kD	18.5 kD
Stress (kPa)*	168+/-51	98+/-15	33+/-7	115+/-56
% Strain*	8+/-3	71+/-13	110+/-9	40+/-15
Slope*	22+/-5	1.32+/-0.31	0.27+/-0.04	2.67+/-0.55

15   *Values are mean+/-S.D.

For the creep tests, eight samples approximately 0.2 X 0.4 X 2 cm were loaded while submersed in saline solution. They were tested with a constant unique predetermined load for one hour and a small recovery load for ten minutes. Gels made from PEG diacrylates of 1 kD, 6 kD, and 10 kD, and PEG tetraacrylates of 18.5 kD PEO molecular weight were used for this study. The 10 kD test was terminated due to a limit error (the sample stretched beyond the travel of the loading frame). The 1 kD sample was tested with a load of 10g and a recovery load of 0.2g. The 6 kD sample was tested at a load of 13g with a recovery load of 0.5g. The 18.5 kD sample was tested at a load of 13g with a recovery load of 0.2g. The choice of loads for these samples produced classical creep curves with primary and secondary regions. The

5   traces for creep for the 1 kD, 6 kD, and 18.5 kD samples appear

in Figure 14A-C, respectively.

Example 20

Water Content of PEO Gels

Solutions of various macromers were made as described

10   above. Gels in the shape of discs were made using a mold.   The

solutions (400 μL) was used for each disc.   The solutions were

irradiated for 2 minutes to ensure thorough gelation.   The disc

shaped gels were removed and dried under vacuum at 60°C for 2

days.   The discs were weighed (WI) and then extracted repeatedly

15   with chloroform for 1 day.   The discs were dried again and

weighed (W2).   The gel fraction was calculated as W2/W1.   This

data appears in Table 7.

Determination of Degree of Hydration

Subsequent to extraction, the discs were allowed to

20   equilibrate with HBS for 6 hours and weighed (W3) after excess

water had been carefully swabbed away.   The total water content

was calculated as (W3-W2) X 100/W3.   The data for gel water

contents is summarized in the following table.

5   TABLE 7

Polymer Coat	% Total Water	% Gel Content
0.4 kD	-	99.8 ± 1.9
1 kD	79.8 ± 2.1	94.5 ± 2.0
6 kD	95.2 ± 2.5	69.4 ± 0.6
10 kD	91.4 ± 1.6	96.9 ± 1.5
18.5 kD	91.4 ± 0.9	80.3 ± 0.9
20 kD	94.4 ± 0.6	85.0 ± 0.4

Example 21

Mechanical Stability of PEO Gels after Implantation

15   PEG diacrylate (10 kD) and PEG tetraacrylate (18.5 kD)
were cast in dogbone shapes as described in Example 19. PEG--
dacrylate or tetraacrylate (23% w/w) in sterile HEPES buffered
saline (HBS) (0.9% NaCl, 10 - HEPES, pH 7.4) containing 900 ppm
of 2,2-dimethoxy-2-phenoxyacetophenone as initiator, was poured
20   into an aluminum mold and irradiated with a LWUV lamp (Black ray)
for 1 min. The initial weights of these samples were found after
oven-drying these gels to constant weight. The samples were
soxhlet-extracted with methylene chloride for 36 hours in order
to leach out any unreacted prepolymer from the gel matrix (sol-
25   leaching) prior to testing. The process of extraction was
continued until the dried gels gave constant weight.

        ICR Swiss male white mice, 6-8 weeks old (Sprague-
Dawley), were anesthetized by an intraperitoneal injection of

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5   sodium pentobarbital. The abdominal region of the mouse was

shaved and prepared with betadine. A ventral midline incision

10-15 mm long was made. The polymer sample, fully hydrated in

sterile PBS (Phosphate buffered saline) or HEPES buffered saline

(for calcification studies), was inserted through the incision

10   and placed over the mesentery, away from the wound site. The

peritoneal wall was closed with a lock stitched running suture

(4.0 silk, Ethicon). The skin was closed with stainless steel

skin staples, and a topical antibiotic (Furacin) was applied over

the incision site. Three animals were used for each time point.

15   One dogbone sample was implanted per mouse and explanted at the

end of 1 week, 3 weeks, 6 weeks, and 8 weeks. Explanted gels

were rinsed in HBS twice and then treated with 0.3 mg/mL pronase

(Calbiochem) to remove any adherent cells and tissue. The

samples were then oven-dried to a constant weight, extracted, and

20   reswelled as mentioned before.

Tensile stress strain test was conducted on both

control (unimplanted) and explanted dogbones in a small

horizontal Instron-like device. The device is an aluminum

platform consisting of two clamps mounted flat on a wooden board

25   between two parallel aluminum guide. The top clamp was

stationary while the bottom clamp was movable. Both the

frictional surfaces of the moving clamp and the platform were

coated with aluminum backed Teflon (Cole-Parmer) to minimize

frictional resistance. The moving clamp was fastened to a device

5   capable of applying a gradually increasing load. The whole set up

       was placed horizontally under a dissecting microscope (Reichert)

       and the sample elongation was monitored using a video camera.

       The image from the camera was acquired by an image processor

       (Argus-10, Hamamatsu) and sent to a monitor. After breakage, a

10   cross section of the break surface was cut and the area measured.

       The load at break was divided by this cross section to find the

       maximum tensile stress. Table 8 lists the stress at fracture of

       PEG tetraacrylate (18.5 kD) hydrogels explanted at various time

       intervals. No significant change in tensile strength was evident

15   with time. Thus, the gels appear mechanically stable to

       biodegradation in vivo within the maximum time frame of implant

       in mice.

TABLE 8

TIME IMPLANTED	STRESS (KPa) (mean ± error*)	STRAIN AV. (mean ± error*)
1 WK	52.8 ± 16.7	0.32 ± 0.19
3 WK	36.7 ± 10.6	0.37 ± 0.17
6 WK	73.3 ± 34.9	0.42 ± 0.26
8 WK	34.1‡	0.30‡
CONTROL	44.9 ± 5.3	0.22 ± 0.22

* Error based on 90% confidence limits.

‡ Single sample.

Example 22

Monitoring of Calcification of PEO Gels

Disc shaped PEG-tetraacrylate hydrogels (m.w. 18.5 kD) were implanted intraperitoneally in mice as mentioned above for a period of 1 week, 3 weeks, 6 weeks, or 8 weeks. Explanted gels were rinsed in HBS twice and treated with Pronase (Calbiochem) to remove cells and cell debris. The samples were then equilibrated in HBS to let free Ca^{++} diffuse out from the gel matrix. The gels were then oven-dried (Blue-M) to a constant weight and transferred to Aluminum oxide crucibles (COORS, high temperature resistant). They were incinerated in a furnace at 700°C for at least 16 hours. Crucibles were checked for total incineration, if any residual remnants or debris was seen they were addition-ally incinerated for 12 hours. Subsequently, the crucibles were filled with 2 mL of 0.5 M HCl to dissolve Ca^{++} salt and other minerals in the sample. This solution was filtered and analyzed with atomic absorption spectroscopy (AA) for calcium content.

Calcification data on PEG-tetraacrylate (mol. wt. 18.5 kD) gel implants is given in Table 9. No significant increase in calcification was observed up to an 8 week period of implantation in mice.

5

TABLE 9

TIME	CALCIFICATION (mean ± error*)
(Days)	(mg Calcium/g of Dry gel wt.)
7	2.33 ± 0.20
21	0.88 ± 0.009
10	42	1.08 ± 0.30
56	1.17 ± 0.26

* Error based on 90% confidence limits.

Example 23

Encapsulation of Neurotransmitter-Releasing Cells

15   Paralysis agitans, more commonly called Parkinson's disease, is characterized by a lack of the neurotransmitter dopamine within the striatum of the brain. Dopamine secreting cells such as cells from the ventral mesencephalon, from neuroblastoid cell lines or from the adrenal medulla can be

20   encapsulated in a manner similar to that of other cells mentioned in prior Examples. Cells (including genetically engineered cells) secreting a precursor for a neurotransmitter, an agonist, a derivative or a mimic of a particular neurotransmitter or analogs can also be encapsulated.

5

Example 24

Encapsulation of Hemoglobin for Synthetic Erythrocytes

Hemoglobin in its free form can be encapsulated in PEG gels and retained by selection of a PEG chain length and cross-link density which prevents diffusion. The diffusion of

10 hemoglobin from the gels may be further impeded by the use of polyhemoglobin, which is a cross-linked form of hemoglobin. The polyhemoglobin molecule is too large to diffuse from the PEG gel. Suitable encapsulation of either native or crosslinked hemoglobin may be used to manufacture synthetic erythrocytes. The entrap-

15 ment of hemoglobin in small spheres (< 5μm) of these highly biocompatible materials would lead to enhanced circulation times relative to crosslinked hemoglobin or liposome encapsulated hemoglobin.

Hemoglobin in PBS is mixed with the prepolymer in the

20 following formulation:

Hemoglobin at the desired amount

PEG DA (MW 10000)	35%
PEG DA (MW 1000)	5%
PBS	60%

25 with 2,2-dimethoxy, 2-phenyl acetophenone at 1.6% of the above solution.

This solution is placed in mineral oil at a ratio of 1 part hemoglobin/prepolymer solution to 5 parts mineral oil and is rapidly agitated with a motorized mixer to form an emulsion.

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5   This emulsion is illuminated with a long-wavelength ultraviolet

light (360nm) for 5 min to crosslink the PEG prepolymer to form a

gel. The mw of the prepolymer may be selected to resist the

diffusion of the hemoglobin from the gel, with smaller PEG DA

molecular weights giving less diffusion. PEG DA of MW 10000,

10   further crosslinked with PEG DA 1000, should possess the

appropriate permselectivity to restrict hemoglobin diffusion, and

it should possess the appropriate biocompatibility to circulate

within the bloodstream.

                        Example 25

              Entrapment of Enzymes for Correction of

15   **Metabolic Disorders and Chemotherapy**

              Congenital deficiency of the enzyme catalase causes

acatalasemia. Immobilization of catalase in PEG gel networks

could provide a method of enzyme replacement to treat this

20   disease. Entrapment of glucosidase can similarly be useful in

treating Gaucher's disease. Microspherical PEG gels entrapping

urease can be used in extracorporeal blood to convert urea into

ammonia. Enzymes such as asparaginase can degrade amino acids

needed by tumor cells. Immunogenicity of these enzymes prevents

25   direct use for chemotherapy. Entrapment of such enzymes in

immunoprotective PEG gels, however, can support successful

chemotherapy. A suitable formulation can be developed for either

slow release or no release of the enzyme.

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Patent

5   Catalase in PBS is mixed with the prepolymer in the following formulation:

           Catalase at the desired amount

           PEG DA (MW 10000)   35%

           PEG DA (MW 1000)   5%

10   PBS   60%

with 2,2-dimethoxy, 2-phenyl acetophenone at 1.6% of the above solution.

           This solution is placed in mineral oil at a ratio of 1 part catalase/prepolymer solution to 5 parts mineral oil and is

15 rapidly agitated with a motorized mixer to form an emulsion. This emulsion is illuminated with a long-wavelength ultraviolet light (360nm) for 5 min to crosslink the PEG prepolymer to form a gel. The mw of the prepolymer may be selected to resist the diffusion of the catalase from the gel, with smaller PEG DA

20 molecular weights giving less diffusion.

           PEG DA of MW 10,000, further crosslinked with PEG DA 1000, should possess the appropriate permselectivity to restrict catalase diffusion, and it should possess the appropriate permselectivity to permit the diffusion of hydrogen peroxide into

25 the gel-entrapped catalase to allow the enzymatic removal of the hydrogen peroxide from the bloodstream. Furthermore, it should possess the appropriate biocompatibility to circulate within the bloodstream.

In this way, the gel is used for the controlled

containment of a bioactive agent within the body. The active

agent (enzyme) is large and is retained within the gel, and the

agent upon which it acts (substrate) is small and can diffuse

into the enzyme rich compartment. However, the active agent is

prohibited from leaving the body or targeted body compartment

because it cannot diffuse out of the gel compartment.

Example 26

Use of PEO Gels as Adhesive to

Rejoin Severed Nerve

A formulation of PEG tetraacrylate (10%, 18.5K), was

used as adhesive for stabilizing the sutureless apposition of the

ends of transected sciatic nerves in the rat. Rats were under

pentobarbital anesthesia during sterile surgical procedures. The

sciatic nerve was exposed through a lateral approach by

deflecting the heads of the biceps femoralis at the mid-thigh

level. The sciatic nerve was mobilized for approximately 1 cm

and transected with iridectomy scissors approximately 3 mm

proximal to the tibial-peroneal bifurcation. The gap between the

ends of the severed nerves was 2-3 mm. The wound was irrigated

with saline and lightly swabbed to remove excess saline.

Sterile, unpolymerized PEG tetraacrylate solution was applied to

the wound. Using delicate forceps to hold the adventitia or

perineurium, the nerve ends were brought into apposition, the

5   macromer solution containing 2,2-dimethoxy-2-phenoxyacetophenone

as a photoinitiator applied to the nerve ends and the wound was

exposed to long wavelength UV-light (365 nm) for about 10 sec to

polymerize the adhesive. The forceps were gently pulled away.

Care was taken to prevent the macromer solution from flowing

10   between the two nerve stumps. Alternatively, the nerve stump

junction was shielded from illumination, e.g., with a metal foil,

to prevent gelation of the macromer solution between the stumps;

the remaining macromer solution was then simply washed away.

       In an alternative approach, both ends of the transected

15   nerve can be held together with one pair of forceps. Forceps

tips are coated lightly with petrolatum to prevent reaction with

the adhesive.

       The polymerized adhesive serves to encapsulate the

wound and adhere the nerve to the underlying muscle. The

20   anastomosis of the nerve ends resists gentle mobilization of the

joint, demonstrating a moderate degree of stabilization. The

muscle and skin were closed with sutures. Re-examination after

one month shows that severed nerves remain reconnected, despite

unrestrained activity of the animals.

Example 27

Surgical Adhesive

Abdominal muscle flaps from female New Zealand white rabbits were excised and cut into strips 1 cm X 5 cm. The flaps were approximately 0.5 to 0.8 cm thick. The lap joint, 1 cm X 1 cm, was made using two such flaps. Two different PEO di- and tetra-acrylate macromer compositions, 0.4K (di-) and 18.5K (tetra-), were evaluated. The 0.4K composition was a viscous liquid and was used without further dilution. The 18.5K composition was used as a 23% w/w solution in HBS. 125 μl of ethyl eosin solution in n-vinyl pyrrolidone (20 mg/ml) along with 50 μl of triethanolamine was added to each ml of the adhesive solution. 100 μl of adhesive solution was applied to each of the overlapping flaps. The lap joint was then irradiated by scanning with a 2 W argon ion laser for 30 seconds from each side. The strength of the resulting joints was evaluated by measuring the force required to shear the lap joint. One end of the lap joint was clamped and an increasing load was applied to the other end, while holding the joint horizontally until it failed. Four joints were tested for each composition. The 0.4K joints had a strength of 12.0 ± 6.9 KPa (mean ± S.D.), while the 18.5K joints had a strength of 2.7 ± 0.5 KPa. It is significant to note that it was possible to achieve photopolymerization and reasonable joint strength despite the 6-8 mm thickness of tissue. A

5   spectrophotometric estimate using 514 nm light showed less than

1% transmission through such muscle tissue.

Example 28

Modification of polyvinyl alcohol

2 g of polyvinyl alcohol (mol wt 100,000-110,000) was

10   dissolved in 20 ml of hot DMSO. The solution was cooled to room

temperature and 0.2 ml of triethylamine and 0.2 ml of acryloyl

chloride was added with vigorous stirring, under an argon

atmosphere. The reaction mixture was heated to 70°C for 2 hr and

cooled. The polymer was precipitated in acetone, redissolved in

15   hot water and precipitated again in acetone. Finally it was

dried under vacuum for 12 hr at 60°C. 5-10% w/v solution of this

polymer in PBS was mixed with the UV photoinitiator and

polymerized using long wavelength UV light to make microspheres

200-1,000 microns in size.

20   These microspheres were stable to autoclaving in water,

which indicates that the gel is covalently cross-linked. The gel

is extremely elastic. This macromer, PVA multiacrylate, may be

used to increase the crosslinking density in PEG diacrylate gels,

with corresponding changes in mechanical and permeability

25   properties. This approach could be pursued with any number of

water-soluble polymers chemically modified with

photopolymerizable groups, for example with water-soluble

polymers chosen from polyvinylpyrrolidone, polyethyloxazoline,

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5   polyethyleneoxide-polypropyleneoxide copolymers, polysaccharides

such as dextran, alginate, hyaluronic acid, chondroitin sulfate,

heparin, heparin sulfate, heparan sulfate, guar gum, gellan gum,

xanthan gum, carrageenan gum, and proteins, such as albumin,

collagen, and gelatin.

10   **Example 29**

           Use of Alternative Photopolymerizable Moieties

           Many photopolymerizable groups may be used to enable

gelation. To illustrate a typical alternative synthesis, a

synthesis for PEG 1K urethane methacrylate is described as

15   follows:

           In a 250 ml round bottom flask, 10 g of PEG 1K diol was

dissolved in 150 ml benzene. 3.38 g of 2-

isocyanatoethylmethacrylate and 20 μl of dibutyltindilaurate were

slowly introduced into the flask. The reaction was refluxed for

20   6 hours, cooled and poured into 1000 ml hexane. The precipitate

was then filtered and dried under vacuum at 60°C for 24 hours.

In this case, a methacrylate free radical polymerizable group was

attached to the polymer via a urethane linkage, rather than an

ester link as is obtained, e.g. when reacting with aryloxyl

25   chloride.

Example 30

Formation of Alginate-PLL-alginate Microcapsules with

Photopolymerizable Polycations

Alginate-polylysine-alginate microcapsules are made by adsorbing, or coacervating, a polycation, such as polylysine (PLL), upon a gelled microsphere of alginate. The resulting membrane is held together by charge-charge interactions and thus has limited stability. To increase this stability, the polycation can be made photopolymerizable by the addition of a carbon-carbon double bond, for example. This can be used to increase the stability of the membrane by itself, or to react, for example, with photopolymerizable PEG to enhance biocompatibility.

To illustrate the synthesis of such a photopolymerizable polycation, 1 g of polyallylamine hydrochloride was weighed in 100 ml glass beaker and dissolved in 10 ml distilled water (DW). The pH of the polymer solution was adjusted to 7 using 0.2 M sodium hydroxide solution. The polymer was then separated by precipitating in a large excess of acetone. It was then redissolved in 10 ml DW and the solution was transferred to 50 ml round bottom flask. 0.2 ml glycidyl methacrylate was slowly introduced into the reaction flask and the reaction mixture was stirred for 48 hours at room temperature. The solution was poured into 200 ml acetone and the precipitate was separated by filtration and dried in vacuum.

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5   This macromer is useful in photochemically stabilizing an

alginate-PLL-alginate, both in the presence or in the absence of

a second polymerizable species such as a PEG diacrylate.

       In addition to use in encapsulating cells in materials

such as alginate, such photopolymerizable polycations may be

10   useful as a primer or coupling agent to increase polymer adhesion

to cells, cell aggregates, tissues and synthetic materials, by

virtue of adsorption of the photopolymerizable polymer bonding to

the PEG photopolymerizable gel.

Example 31

Cellular Microencapsulation for Evaluation of

Anti-Human Immunodeficiency Virus Drugs In Vivo

15   HIV infected or uninfected human T-lymphoblastoid cells

can be encapsulated into PEG gels as described for other cells

above. These microcapsules can be implanted in a nonhuman animal

20   and then treated with test drugs such as AZT or DDI. After

treatment, the microcapsules can be harvested and the

encapsulated cells screened for viability and functional normalcy

using a fluorescein diacetate/ethidium bromide live/dead assay.

Survival of infected cells indicates successful action of the

25   drug. Lack of biocompatibility is a documented problem in this

approach to drug evaluations, but the highly biocompatible gels .

described herein solve this problem.

5 **Example 32**

Use of Alternative

Photoinitiator/Photosensitizer Systems

It is possible to initiate photopolymerization with a
wide variety of dyes as initiators and a number of electron
10 donors as effective cocatalysts. Table 10 illustrates
photopolymerization initiated by several other dyes which have
.chromophores absorbing at widely different wavelengths. All
gelations were carried out using a 23% w/w solution of 18.5 kD
PEG tetraacrylate in HEPES buffered saline. These initiating
15 systems compare favorably with conventional thermal initiating
systems, as can also be seen from Table 10.

·Table 10

Polymerization Initiation

INITIATOR	LIGHT SOURCE*	TEMPERATURE °C	APPROXIMATE GEL TIME, (SEC)
Eosin Y, 0.00015M, Triethanolamine 0.65M	S1 with UV filter	25	10
Eosin Y, 000015M; Triethanolamine 0.65M	S4	25	0.1
Methylene Blue, 0.00024M; p-toluenesulfonic acid, 0.0048M	S3	25	120
2,2-dimethoxy-2-phenyl acetophenone 900 ppm	S2	25	8
Potassium Persulfate 0.0168M	-	75	180
Potassium Persulfate 0.0168M; tetramethyl ethylene-diamine 0. 039M	-	25	120
Tetramethyl ethylene-diamine 0.039M; Riboflavin 0.00047M	S1 with UV filter	25	300

*   LIST OF LIGHT SOURCES USED

CODE	SOURCE
S1	Mercury lamp, LEITZ WETZLER Type 307-148.002, 100W
S2	Black Ray longwave UV lamp, model B-100A W/FLOOD
S3	MELLES GRIOT He-Ne laser, 10mW output, 1=632 nm
S4	American laser corporation, argon ion laser, model 909BP-15-01001; 1=488 and 514 nm

Numerous other dyes can be used for

photopolymerization. These dyes include but are not limited to

Erythrosin, phloxime, rose bengal, thionine, camphorquinone,

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ethyl eosin, eosin, methylene blue, and riboflavin. Possible

cocatalysts that can be used include but are not limited to: N-

methyl diethanolamine, N,N-dimethyl benzylamine, triethanolamine,

triethylamine, dibenzyl amine, N-benzyl ethanolamine, N-isopropyl

benzylamine.

BIODEGRADABLE MACROMERS

Table 11 shows the code names of the various macromers

synthesized in or for use in the examples, along with their

composition in terms of the molecular weight of the central PEG

segment and the degree of polymerization of the degradable

comonomer.

Table 11: Macromer Molecular Weight and Composition

PEG molecular	Comonomer	D.P. of conomoner per OH group	Polymer weight Code
20,000	glycolide	15	20KG
18,500	glycolide	2.5	18.5K
10,000	glycolide	7	10KG
6,000	glycolide	5	6KG
4,000	glycolide	5	4KG
1,000	glycolide	2	1KG
20,000	DL-lactide	10	20KL
18,500	DL-lactide	10	18.5KL
10,000	DL-lactide	5	10KL
6,000	DL-lactide	5	6KL
1,000	DL-lactide	2	1KL
600	DL-lactide	2	0.6KL
600	DL-lactide + caprolactone(CL)	lactide 2; CL 1	0.6KLCL
18,500	caprolactone	2.5	18.5KCL
18,500	–	–	18.5KCO

Example 33

Synthesis of Photopolymerized Biodegradable Hydrogels.

PEG-based hydrogels

PEG-based biodegradable hydrogels are formed by the rapid laser or UV photopolymerization of water soluble macromers. Macromers, in turn, are synthesized by adding glycolic acid oligomers to the end groups of PEG and then capping with acrylic end groups. The PEG portions of the macromers confer water solubility properties, and subsequent polymerization results in cell-nonadhesive hydrogels. Glycolic acid oligomers serve as the hydrolyzable fraction of the polymer network, while acrylic end

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5   groups facilitate rapid polymerization and gelation of the

       macromers.

              In preparation for synthesis, glycolide (DuPont) or DL-

       lactide (Aldrich) was freshly recrystallized from ethyl acetate.

       PEG oligomers of various molecular weight (Fluka or Polysciences)

10   were dried under vacuum at 110°C prior to use. Acryloyl chloride

       (Aldrich) was used as received. All other chemicals were of

       reagent grade and used without further purification.

              Macromer synthesis

              A 250 ml round bottom flask was flame dried under

15   repeated cycles of vacuum and dry argon. 20 gm of PEG (molecular

       weight 10,000), 150 ml of xylene and 10 μgm of stannous octoate

       were charged into the flask. The flask was heated to 60°C under

       argon to dissolve the PEG and cooled to room temperature. 1.16

       gm of glycolide was added to the flask and the reaction mixture

20   was refluxed for 16 hr. The copolymer was separated on cooling

       and was recovered by filtration. This copolymer was separated on

       cooling and recovered by filtration. This copolymer (10K PEG-

       glycolide) was used directly for subsequent reactions. Other

       polymers were similarly synthesized using DL-lactide or ε-

25   caprolactone in place of glycolide and using PEG of different

       molecular weights.

              Synthesis of photosensitive oligomers (macromers):

              19 gm of 10K PEG-glycolide copolymer was dissolved in

       150 ml methylene chloride and refluxed with 1 ml acryloyl

                              - 93 -

5   chloride and 1.2 ml of triethylamine for 12 hr under an argon

atmosphere. The solid triethylamine hydrochloride was separated

by filtration and the polymer was precipitated by adding the

filtrate to a large excess of hexane. The polymer (capped by an

acrylate at both ends) was further purified by repeated

10   dissolution and precipitation in methylene chloride and hexane

respectively.

Table 12 lists certain macromers synthesized. The

degree of polymerization of the glycolide chain extender was kept

low so that all polymers have approximately 10 ester groups per

15   chain, or about 5 per chain end. When these polymers are

photopolymerized, a crosslinked three-dimensional network is

obtained. However, each chain segment in the resulting network

needs just one ester bond cleaved at either end to "degrade."

These ester cleavages enable the chain to dissolve in the

20   surrounding physiological fluid and thereby be removed from the

implant site. The resulting hydrolysis products, PEG and

glycolic acid, are water soluble and have very low toxicity.

5

TABLE 12

Macromers Synthesized

Polymer Code	Mol. Wt. Of Central PEG Chain (daltons)	% Glycolide in Extremities	% ∈-Caprolactone in Extremities	Calculated Mol. Wt. Of Extremities (daltons)	Appearance
0.4K	400	100	--	580	Viscous liquid
1KG	1000	100	--	300	Viscous liquid
4KG	4000	100	--	232	White solid
10KG	10000	100	--	580	White solid
18.5KG	18500	100	--	1160	Yellow solid
co18.5KGCL	18500	50	--	580	White solid

Due to the presence of only a few units of glycolic acid per oligomeric chain, the solubility properties of the photocrosslinkable prepolymers are principally determined by the central PEG chain. Solubility of the macromers in water and methylene chloride, both of which are solvents for PEG, is not adversely affected as long as the central PEG segment has a molecular weight of 1,000 daltons or more. Solubility data for the prepolymers synthesized is given in Table 13.

Table 13: SOLUBILITY DATA

Solvent	1KG	4KG	10KG	18.5KG	TMP*
DMSO	–	■	–	■	■
Acetone	–	■	■	■	–
Methanol	–	■	–	■	–
Water	–	–	–	–	■
Hexane	■	■	■	■	■
Methylene Chloride	–	–	–	–	–
Cold Xylene	■	■	■	■	–
Hot Xylene	–	–	–	–	–
Benzene	■	■	■	■	–

–   Soluble

■   Not Soluble

*   Trimethylolpropane glycolide triacrylate

PEG chains with different degrees of polymerization of DL-lactide were synthesized to determine the degree of substitution for which water solubility of the macromers can be retained. The results are shown in Table 14. Beyond about 20% substitution of the hydrophilic PEG chain with hydrophobic DL-lactoyl or acrylate terminals leads to the macromers becoming insoluble in water, though they are still soluble in organic solvents such as methylene chloride.

Table 14: Solubility of Macromers

D.P.* of Ethylene Oxide	D.P.* of lactide or glycolide	% extension of PEG chain	Solubility in water
420	4	0.1	soluble
420	10	2.4	soluble
420	20	4.8	soluble
420	40	9.5	soluble
420	80	19	insoluble
23	2	8.7	soluble
23	4	17.4	soluble
23	10	43.5	insoluble
23	40	174	insoluble
5	4	80	insoluble
10	4	40	soluble

* degree of polymerization

Photopolymerization

The macromers can be gelled by photopolymerization using free radical initiators, with the presence of two acrylic double bonds per chain leading to rapid gelation. A 23% w/w solution of various degradable polymers in HEPES buffered saline containing 3 μl of initiator solution (300 mg/ml of 2,2-dimethoxy-2-phenyl-acetophenone in n-vinyl pyrrolidone) was used. 100 μl of the solution was placed on a glass coverslip and irradiated with a low intensity long wavelength UV (LWUV) lamp (Blak-Ray, model 3-100A with flood). The times required for gelation to occur were noted and are given below. These times are typically in the range of 10 seconds. This is very significant because these reactions are carried out in air (UV

5   initiated photopolymerizations are slow in air as compared to an

inert atmosphere) and using a portable, low powered long wave UV

(LWUV) emitting source. Oxygen, which often inhibits free

radical reactions by forming species which inhibit propagation,

did not seem to slow down the polymerization. Such fast

10   polymerizations are particularly useful in applications requiring

in situ gelations. This rapid gelation is believed to be due to

the formation of micelle-like structures between the relatively

hydrophobic polymerizable groups on the macromer, thereby

increasing the local concentration of the polymerizable species

15   in aqueous solution and increasing polymerization rates.

Visible laser light is also useful for polymerization.

Low intensity and short exposure times make visible laser light

virtually harmless to living cells since the radiation is not

strongly absorbed in the absence of the proper chromophore.

20   Laser light can also be transported using fiber optics and can be

focused to a very small area. Such light can be used for rapid

polymerization in highly localized regions; gelation times for

selected prepolymers are given in Table 15. In each case, 0.2 ml

of a 23% w/v photosensitive oligomer solution is mixed with ethyl

25   eosin (10^{-4} M) and triethanol amine (0.01 to 0.1 M) and the

solution is irradiated with an argon ion laser (American argon

ion laser model 905 emitting at 514 nm) at a power of 0.2-0.5

W/cm^2. The beam is expanded to a diameter of 3 mm and the sample

is slowly scanned until gelation occurs.

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Table 15: Gelation Times

Polymer	UV polymerization * gelation time (mean ±S.D.) (s)	Laser Polymerization** gelation time (s)
1KG	5.3±4.1	<1
4KG	14.7±0.5	<1
6KG	9.3±0.5	<1
10KG	18.±0.8	<1
10KL	7.7±0.5	<1
18KG	23.3±1.2	<1
20KG	13.3±0.5	<1

* Initiator: 2,2-dimethoxy-2-phenylacetophenone, concentration 900 ppm: 0.2 ml of 23% monomer solution in PBS
** Argon ion laser emitting at 514nm. power 3 W/cm^2: ethyloeosin, triethanol amine initiating system: 0.2 ml of 23% monomer solution in PBS

Biodegradability

Biodegradation of the resulting polymer network is an important criteria in many biomedical applications. Degradation of poly(glycolic acid and poly(DL-lactic acid) has been well documented in the literature. The degradation mainly takes place through the hydrolysis of the ester bond; the reaction is second order and highly pH dependent. The rate constant at pH 10 is 7 times faster than that at pH 7.2.

Such facile biodegradation is surprising because poly(α-hydroxyacidesters) are hydrophobic and highly insoluble in water. Accessibility of the polymer matrix to the aqueous

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5   surrounding is therefore limited. However, because the networks

       are hydrogels which are swollen with water, all the ester

       linkages in the network are in constant contact with water with

       the aqueous surroundings. This results in a uniform bulk

       degradation rather than a surface degradation of these gels.

10   Table 16 gives hydrolysis data for some of these

       networks; times listed are for complete dissolution of 60 mg of

       gel at pH 7.2 and 9.6. As noted, most of the gels dissolve

       within 12 hours at pH 9.6. 18.5k gel dissolves within 2.5 hr at

       pH 9.6 whereas 18.5KCO gel does not dissolve in 3 days,

15   indicating that the lactoyl, glycoloyl, or ε-caprolactoyl ester

       moiety is responsible for degradation of these networks. It also

       can be seen that the 18.5KG gel hydrolyzes more rapidly than the

       4KG gel. This may be due to the reduced hydrophilicity and

       higher crosslink density of the latter gel.

20   **Table 16: Hydrolysis Data**

Oligomer used for gelation	Time taken to dissolve gel at pH 9.6 (h)	Time taken to dissolve gel at pH 7.2 (days)
4KG	6.2	5.5
10KG	12.25	5.5
18.5KG	2.25	>7
18.5KCL	>5 days	>7
18.5KCO	>5 days	>7

5   Characterization of macromers

FTIR spectra of the prepolymers were recorded on a

DIGILAB model FTS 15/90. The absorption at 1110 cm^{-1}

(characteristic C-O-C absorption of PEG) shows the presence of

PEG segments. The strong 1760 cm^{-1} absorption shows the presence

10   of glycolic ester. The absence of hydroxyl group absorption

around 3400 cm^{-1} and a weak acrylic double bond absorption at

1590 cm^{-1} shows the presence of acrylic double bonds at the end

groups.

500 MHz proton and 125 MHz carbon-13 spectra were

15   recorded on a GE 500 instrument. The presence of a very strong

peak at 4.9 ppm due to CH$_2$ methylene from the PEG segment, a peak

at 5.09 ppm due to the glycolic ester segment and an acrylic

proton singlet at 5.8 ppm can be easily seen from proton NMR.

The estimated molecular weight of PEG segment and glycolic acid

20   segment for different copolymers is shown in Table 12. The

carbonyl peak at 169.39 ppm from glycolic acid and 36.5 ppm peak

from methylene carbons from PEG in carbon-13 NMR are consistent

with the reported chemical composition of these copolymers.

Differential scanning calorimetry (Perkin Elmer DSC-7)

25   was used to characterize the oligomers for thermal transitions.

The oligomers were heated from -40°C to 200°C at a rate of

20°C/min, presumably causing polymerization. The polymer was

then cooled to -40°C at a rate of 60°C/min and again heated to

200°C at a rate of 20°C/min. The first scans of biodegradable

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18.5K PEG glycolide tetraacrylate (18.5KG) oligomer were compared

to that of the non-degradable 18.5K PEG tetraacrylate (18.5KCO)

scan. It was seen that a glass transition appears in the 18.5KG

at -2°C while no such transition exists in the 18.5KCO. A small

melting peak at 140°C was also evident due to the few glycolic

acid mers which can crystallize to a limited extent. The melting

peak for PEG is shifted downwards in 18.5KG to 57°C from 60.7°C

for 18.5KCO. This is probably due to disturbance of the PEO

crystalline structure due to the presence of the glycolic acid

linkages. In the third cycle, by which time the oligomers have

presumably polymerized, the Tg and Tm transitions for the

glycolide segments can no longer be seen, indicating that a

crosslinked network has formed and the glycolic acid segments are

no longer capable of mobility.

The degree of polymerization (D.P.) of the degradable

segments added to the central water soluble PEG chain was

determined in several cases using ^{1}H NMR. The experimentally

determined D.P. was seen to be in good agreement with the

calculated number, as shown by Figure 15. Thus, the ring opening

reaction initiated by the PEG hydroxyls proceeds to completion,

giving quantitative yields.

<u>Determination of Total Water, Free Water Bound Water</u>

Solutions of various degradable macromers were made as

described above. Gels in the shape of discs were made using a

mold. 400 μl of solution was used for each disc. The solutions

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5   were irradiated for 2 minutes to ensure thorough gelation. The

disc shaped gels were removed and dried under vacuum at 60°C for

2 days. The discs were weighed (W1) and then extracted

repeatedly with chloroform for 1 day. The discs were dried again

and weighed (W2). The gel fraction was calculated as W2/W1.

10   This data appears in Table 17.

Subsequent to extraction, the discs were allowed to

equilibrate with PBS for 6 hours and weighed (W3 after excess

water had been carefully swabbed away). The total water content

was calculated as (W3-W2) X 100/W3. Differential scanning

15   calorimetry (DSC) was used to determine the amount of free water

that was available in the gels. A scan rate of 20°C/min was used

and the heat capacity for the endotherm for water melting was

measured (H1). The heat capacity of HBS was also measured (H2).

The fraction of free water was calculated as H1/H2. The residual

20   water was assumed to be bound due to hydrogen bonding with the

PEO segments. The presence of free water in the gels was

indicated. This free water can be expected to help proteins and

enzymes entrapped in such gels in maintaining their native

conformation and reducing deactivation. Thus these gels would

25   appear to be suited for controlled release of biological

micromolecules. The data for gel water content is summarized in

Table 17.

Table 17: Hydrogel Water content

Polymer Code	% Free Water	% Bound Water	% Total Water	% Gel Content
1KG	68.4	14	82.3±2.6	61.3±5.2
4KG	78.0	9.3	87.3±1.8	56.3±0.9
6KG	74.8	13.4	88.1±3.3	66.5±2.35
10KG	83.7	10.8	94.5±0.5	54.3±0.6
10KL	82.0	9.7	91.7±0.5	63.9±3.7
18.5KG	71.8	22.3	94.0±0.4	47.0±4.9
20KG	79.8	14.8	94.5±0.4	44.5±4.8

Example 34

Use of multifunctional macromers.

30 g of a tetrafunctional water soluble PEG (MW 18,500) (PEG 18.5k) was dried by dissolving the polymer in benzene and distilling off the water benzene azeotrope. In a glove bag, 20 g of PEG 18.5 k, 1.881 g of glycolide and 15 mg of stannous octoate were charged into a 100 ml round bottom flask. The flask was capped with a vacuum stopcock, placed into a silicone oil bath and connected to a vacuum line. The temperature of the bath was raised to 200°C. The reaction was carried out for 4 hours at 200°C and 2 hours at 160°C. The reaction mixture was cooled, dissolved in dichloromethane and the copolymer was precipitated by pouring into an excess of dry ethyl ether. It was redissolved in 200 ml of dichloromethane in a 500 ml round bottom flask cooled to 0°C. To this flask, 0.854 g of triethylamine and 0.514 ml of acryloyl chloride were added under nitrogen atmosphere and the reaction mixture was stirred for 12 h. at 0°C. The triethyl

5   amine hydrochloride was separated by filtration and the copolymer
was recovered from filtrate by precipitating in diethyl ether.
The polymer was dried at 50°C under vacuum for 1 day.

Example 35

Synthesis of a photosensitive macromer containing DL-lactide

10   PEG (MW) 20,000) (PEG 20k) was dried by dissolving in
benzene and distilling off the water benzene azeotrope. In a
glove bag, 32.43 g of PEG 20k, 2.335 g of DL-lactide and 15 mg of
stannous octoate were charged into a 100 ml round bottom flask.
The flask was capped with a vacuum stopcock, placed into a
15   silicone oil bath and connected to a vacuum line. The
temperature of the bath was raised to 200°C. The reaction was
carried out for 4 hours at 200°C. The reaction mixture was
cooled, dissolved in dichloromethane and the copolymer was
precipitated by pouring into an excess of dry ethyl ether. It
20   was redissolved in 200 ml of dichloromethane in a 500 ml round
bottom flask cooled to 0°C. To this flask, 0.854 g of
triethylamine and 0.514 ml of acryloyl chloride were added under
nitrogen atmosphere and the reaction mixture was stirred for 12
hours at 0°C. The triethyl amine hydrochloride was separated by
25   filtration and the copolymer was recovered from filtrate by
precipitating in diethyl ether. The polymer was dried at 50°C
under vacuum for 1 day.

Example 36

Synthesis of a Photosensitive Precursor

Containing DL-Lactide and ε-Caprolactone.

PEG (MW 600) (PEG 0.6k) was dried by dissolving in benzene and distilling off the water benzene azeotrope. In a glove bag, 0.973 g of PEG 0.6k, 0.467 g of DL-lactide along with 0.185 g of ε-caprolactone and 15 mg of stannous octoate were charged into a 50 ml round bottom flask. The flask was capped with a vacuum stopcock, placed into a silicone oil bath and connected to a vacuum line. The temperature of the bath was raised to 200°C. The reaction was carried out for 4 hours at 200°C and 2 hours at 160°C. The reaction mixture was cooled, dissolved in dichloromethane and the copolymer was precipitated by pouring into an excess of dry ethyl ether. It was redissolved in 50 ml of dichloromethane in a 250 ml round bottom flask cooled to 0°C. to this flask, 0.854 g of triethylamine and 0.514 ml of acryloyl chloride were added under nitrogen atmosphere and the reaction mixture was stirred for 12 hours at 0°C. The triethyl amine hydrochloride was separated by filtration and the copolymer was recovered from filtrate by precipitating in diethyl ether. The polymer was dried at 50°C under vacuum for 1 day and was a liquid at room temperature.

5   **Example 37**

           Selection of dyes for use in photopolymerization

           It is possible to initiate photopolymerization with a

wide variety of dyes as initiators and a number of electron

donors as effective cocatalysts. Table 18 illustrates

10   photopolymerization initiated by several other dyes which have

chromophores absorbing at widely different wavelengths. All

gelations were carried out using a 23% w/w solution of 18.5KG in

HEPES buffered saline. These initiating systems compare

favorably with conventional thermal initiating systems, as can

15   also be seen from Table 18. Other photoinitiators that may be

particularly useful are 2-methoxy-2-phenyl acetophenone and

camphorquinone.

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Table 18:   Polymerization Initiation of 18.5KG PEG

INITIATOR	LIGHT SOURCE*	TEMPERATURE °C	TIME (SEC)	GEL
Eosin Y, 0.00015M; Triethanolamine 0.65M	S1 with UV filter	25		10
Eosin Y, 0.00015M; Triethanolamine 0.65M	S4	25		0.1
Methylene Blue, 0.00024M; p-toluenesulfinic acid, 0.0048M	S3	25		120
2,2-dimethoxy-2-phenyl acetophenone 900 ppm	S2	25		8
Potassium persulfate 0.0168M	-	75		180
Potassium Persulfate 0.0168M; tetramethyl ethylene-diamine 0.039M	-	25		120
Tetramethyl ethylene-diamine 0.039M; Riboflavin 0.00047M	S1 with UV filter	25		300

*LIST OF LIGHT SOURCES USED

CODE   **SOURCE**

S1   Mercury lamp, LEITZ WETSLER Type 307-148.002,   100W
S2   Black Ray longwave UV lamp, model B-100A
          W/FLOOD
S3   MELLES GRIOT He-Ne laser, 10mW output, 1=632
          nm
S4   American laser corporation, argon ion laser,
          model 909BP-15-01001; λ=488 and 514 nm

          Numerous other dyes can be used for

photopolymerization. These dyes include but are not limited to:

Erythrosin, phloxine, rose bengal, thioneine, camphorquinone,

ethyl eosin, eosin, methylene blue, and riboflavin. The several

possible cocatalysts that can be used include but are not limited

to: N-methyl diethanolamine, N,N-dimethyl benzylamine,

5   triethanol amine, triethylamine, dibenzyl amine, N-benzyl

ethanolamine, N-isopropyl benzylamine, and N-vinyl pyrrolidinone.

Example 38

Thermosensitive Biodegradable Gels from N-Isopropyl Acrylamide

<u>Synthesis of low molecular weight polyisopropyl acrylamide</u>

10   N-isopropyl acrylamide (NIPAAm) was recrystallized from

65:35 hexane benzene mixture. Azobisisobutyronitrile (AIBN) was

recrystallized from methanol. 1.5 g of NIPAAm was polymerized

using 3 mg of AIBN and 150 mg of mercaptoethanol in 1:1 acetone

water mixture (24 hours at 65°C). The viscous liquid after

15   polymerization was purified by dissolving in acetone and

precipitating in diethyl ether. Yield 80%.

This hydroxy terminated low molecular weight

poly(NIPAAm) was used in chain extension reactions using

glycolide and subsequent endcapping reaction using acryloyl

20   chloride as described in other examples.

1 g of modified poly(NIPAAm) based oligomer and 0.2 g

1KL were dissolved in water at 0°C and polymerized at 0°C using

2-2-dimethoxy-2-phenylacetophenone (900 PPM).

5   **Example 39**

In Vitro **Degradation**

The gels were extracted as described in Example 32 to

remove the unpolymerized macromer fraction fraction and the gels

were then placed in 50 mM HEPES buffered saline (0.9% NaCl), pH

10   7.4 at 37°C. Duplicate samples were periodically removed, washed

with fresh HBS and dried at 100°C for 1 day and weighed to

determine mass loss in the gel. The compositions of the various

gels used were the same as described in the previous examples.

Table 19 shows the extent of degradation of these gels given as

15   percent of mass lost over time. The respective times are given

in parenthesis along with the mass loss data.

Table 19: Gel Degradation

1KG	20.1% (1 d), 20.36±0.6 (2d), 21.7± (6d), 28.8±16.6 (10 d) estimated total Degradation time 45 days.
4KG	38.9 (1d), 60.3±4.2 (2d), 78.9 (3d), 99.3±4.7 (6d). Total degradation time 5.5 days.
6KG	18.3±6.8 (1d), 27.4±1.0 (2d), 32.8±11.3 (3d), 104.8±3.2 (5d). total degradation time 4.5 days 10KG 0.6±0.6 (8 hr), 100 (1d). Total degradation time 1 day.
10KL	10.0±4.84 (2d), 6.8±1.7 (3d), 4.5±3.1 (6d), 8.0±0.2 (10d). Total degradation time estimated to be 20 days.
20KG	68.1±4.2 (8hr), 99.7±0.3 (1d). Total degradation time 15 hr.

5   **Example 40**

                  Fibroblast adhesion and spreading

          The *in vitro* response of Human foreskin fibroblast

    (HFF) cells to photopolymerized gels was evaluated through cell

    culture on polymer networks. 0.2 ml of monomer solution was UV

10 polymerized on an 18 x 18 mm glass coverslips under sterile

    conditions. HFF cells were seeded on these gels at a cell

    density of 1.8×10^4 cells/sq cm of coverslip area in Dulbecco's

    Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf

    serum. The gels were incubated for 6 hr at 37°C in a 5% CO_2

15 environment, at the end of which they were washed twice with

    phosphate buffered saline (PBS). The adherent cells were fixed

    using a 2% glutaraldehyde solution in PBS. The gels were

    examined under a phase contrast microscope at a magnification of

    200X, and the number of adherent and spread cells evaluated by

20 examining five fields selected at predetermined locations on the

    coverslips.

          The number of adherent cells is reported in Table 20

    along with those for glass control surfaces. Cell adhesion is

    seen to be dramatically lowered on gel-coated glass.

5   **Table 20: Cell Adhesion**

Surface	Attached Cells/cm^2
glass	13220±3730
18.5KG	250±240
18.5KCL	1170±1020
18.5KCO	390±150

Typical photographs of these cells on the 18.5KCL gel
surfaces and on control glass surfaces are shown in Figures 16A
and 16B. It can be easily seen from Table 20 that these gels are
highly resistant to cellular growth. Even the 18.5KCL is still
less than 10% of the glass. Cells attached to the glass surface
show a flattened and well-spread morphology whereas the few cells
that are attached to the gel are rounded and loosely attached.
This may result from the fact that hydrated PEG chains have a
high motility and have been shown to be effective in minimizing
protein adsorption. One of the mechanisms by which cell adhesion
is mediated is through the interaction of cell surface receptors
with adsorbed cell adhesion proteins. Thus the reduction in
overall protein adsorption results in minimal cell adhesion
protein adsorption and reduced cell adhesion.

25   **Example 41**

      Release of Protein (Bovine Serum Albumin) from Polymers

            1KG was used for this study. This macromer was liquid
at room temperature and was used as such. 1 mg of bovine serum
albumin (BSA) was added per ml of monomer solution along with 0.9

5   mg/ml of 2,2-dimethoxy-2-phenyl-acetophenone as initiator. The

protein was dissolved in the monomer solution and disc shaped

gels were made by exposing 0.2 g of macromer mixture to LWUV for

1 min. Two such discs were placed in a flask containing 20 ml of

PBS and incubated at 37°C. Two aliquots of 20 μl each were

10   removed from these flasks periodically and the amount of BSA

released was assayed using the Bio-Rad total protein assay. The

release profile for BSA is shown in Figure 17A. It can be seen

that the release of BSA is relatively steady over more than a

month.

                              Example 42

                          Enzyme Release Assay

15   Water solubility of the macromers means gelation can be

carried out in a non-toxic environment. This makes these

materials suitable for intraoperative uses where *in situ* gelation

20   is needed. Since the precursors are water soluble, the gels can

be used as drug delivery vehicles for water soluble drugs,

especially macromolecular drugs such as enzymes, which would

otherwise be denatured and lose their activity. Release of

lysosome and tPA from the polymers was used to illustrate the

25   feasibility of using biodegradable hydrogels for controlled

release of biomolecules.

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5   ## Lysozyme release

The enzyme lysozyme (MW:14,400) is a convenient model
for release of a low molecular weight protein from a
biodegradable gel. The Biorad total protein assay was used to
quantify the enzyme released. The enzyme was dissolved in PBS at

10   a concentration of 20 mg/ml. The monomer PEG-dl-lactic acid-
diacrylate was dissolved in PBS to produce a 40% solution. The
lysozyme solution was added to the monomer solution to attain a
24% monomer solution. The monomer/lysozyme solution was
polymerized under UV in a cylindrical mold, using 30 μl of the

15   initiator 2,2-dimethoxy-2-phenyl-acetophenone in 1-vinyl-2-
pyrrolidone (30 mg/ml) as the initiator. The polymer was cut
into 10 equal sized pieces and immersed in 10 ml PBS. Samples of
the PBS were withdrawn at intervals and assayed for lysozyme
released into the PBS. Lysozyme was released from the PEG-DL-

20   lactic acid-diacrylate gel over an 8 day interval, with the
maximum rate of release occurring within the first 2 days, as
shown by Figure 17B.

Release of recombinant t-PA

Three macromers were used for these studies: 1KL, 4KG,

25   and 18.5KG. The 1KL macromer was liquid at room temperature and
was used as such. The second macromer, 4KG, was used as a 75%
w/w solution in PBS. The third composition was a mixture of
equal parts of 1KL and a 50% w/w solution of 18.5KG. 3.37 mg of
tissue plasminogen activator (single chain, recombinant, M.W.

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5   71,000) was added per gram of macromer solution along with 0.9

mg/ml of 2,2 dimethoxy 2 phenyl acetophenone as initiator. The

protein was dissolved with the macromer and disc shaped gels were

made by exposing 0.2 g of macromer mixture to LWUV for 1 minute.

Two such discs were rinsed with PBS, placed in a flask containing

10   5 ml of PBS and incubated at 37°C. Two aliquots of 100 μl each

were removed from these flasks periodically and the amount of

active t-PA released was assayed using a chromogenic substrate

assay (Kabi-vitrum). The release profiles from the 1K lactide

gels, 4K glycolide gels, and the 50/50 1K glycolide/18.5K

15   glycolide are shown in Figures 18A - 18C. Fully active tPA can

be released for periods up to at least two months.

By selecting an appropriate formulation, the release

rate can be tailored for a particular application. It is also

possible to combine formulations with different molecular weights

20   so as to synergistically achieve appropriate attributes in

release and mechanical characteristics.

For prevention of postoperative adhesions, in addition

to the barrier effect of the gels, the gels can be loaded with a

fibrinolytic agent to lyse incipient filmy adhesions which escape

25   the barrier effect. This further enhances the efficacy of

biodegradable gels in adhesion prevention.

5   **Example 43**

Toxicity of Polymers and Commercial Adhesives

To evaluate the toxicity of· *in situ* polymerization of

the macromer solutions described herein, as compared to

commercial adhesives, 100 μl of 18.5KCO prepolymer solution was

10   placed on the right lobe of a rat liver and gelled by exposing it

to LWUV for 15 sec; similarly, a few drops of a n-butyl

cyanoacrylate based glue were placed on the left lobe. The liver

was excised after a week, fixed in 10% neutral buffered formalin,

blocked in paraffin, sectioned and stained using hematoxylin and

15   eosin.

No adverse tissue reaction was evident on the surface

of the lobe exposed to the biodegradable gel. No inflammatory

reaction to the polymerization process can be seen. The

epithelium looks normal, with no foreign body reaction.

20   In comparison, the lobe exposed to cyanoacrylate glue

shows extensive tissue necrosis and scarring with 10-30 cell deep

necrotic tissue. Fibrosis is evident in the necrotic portions

close to underlying normal tissue.

                           Example 44

25   **Prevention of Post-Surgical Adhesions with Photopolymerized**

Biodegradable Polymer

A viscous sterile 23% solution in phosphate buffered

saline (8.0 g/l NaCl, 0.201 g/l KCl, 0.611 g/l Na_2HPO_4, 0.191 g/l

                          - 116 -

5   KH_2PO_4, pH 7.4) of polyethylene glycol (M.W. 18,500) which has

been chain extended on both ends with a short polyglycolide

repeat unit (average number of glycolidyl residues: 10 on each

end) and which has been subsequently terminated with an acrylate

group was prepared. Initiator needed for the crosslinking

10   reaction, 2,2-dimethoxy-2-phenyl acetophenone, was added to the

macromer solution to achieve an initiator concentration of 900

ppm. A 30 second exposure to a long wave UV lamp (Blak Ray) is

sufficient to cause polymerization.

Animal models evaluated

15   Animal models evaluated included a rat cecum model and

a rabbit uterine horn model. In the rat cecum mode, 6 out of 7

animals treated with the macromer solution showed no adhesions

whatsoever, while untreated animals showed consistent dense

adhesion formation. In the rabbit uterine horn model, a

20   significant (p<0.01) reduction in adhesion formation was seen in

the animals treated with the gel. Studies conducted in rats

using only the ungelled viscous precursor solution (no LWUV)

failed to prevent the formation of adhesions.

Rat cecum model

25   Twenty-one Sprague Dawley male rats having an average

weight of 250 gm were divided into three groups for treatment and

two for controls. The abdomen was shaved and prepared with a

betadine solution. A midline incision was made under Equithesin

anesthesia. The cecum was located and 4 to 5 scrapes were made

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5   on a region about 2 x 1 cm on one side of the cecum, using a 4 x

4 in gauze pad to produce serosal injury and punctate bleeding.

The abdominal incisions in these animals were closed using a

continuous 4-0 silk suture for the musculoperitoneal layer and

7.5 mm stainless steel staples for the cutaneous layer. A

10   topical antibiotic was applied at the incision site.

The first group consisted of 7 animals serving as

controls without treatment, to confirm the validity of the model.

The second group served as a control with the application of the

precursor but without photopolymerization to form the hydrogel.

15   After induction of the cecal injury, about 0.25 ml of the

precursor solution was applied to the injury site using a pipet.

The abdominal incision was then closed as above.

The third group served as the gel treatment group and

was prepared as the second group except that the precursor film

20   was exposed to a LWUV lamp for 45 seconds to cause gelation.

Both the obverse and reverse sides of the cecum were similarly

treated with precursor and light. No attempt was made to dry the

surface of the tissue, to remove blood, or to irrigate the area

prior to treatment.

25   The animals were sacrificed at the end of two weeks by

CO_2 asphyxiation. The incisions were reopened and adhesions were

scored for location, extent, and tenacity. The extent of

adhesions was reported as a percentage of the traumatized area of

the cecum which forms adhesions with adnexal organs or the

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5   peritoneal wall. Tenacity of the adhesions was scored on a scale

from 0 to 4: no adhesions - grade 0; tentative transparent

adhesions which frequently separate on their own - grade 1;

adhesions that give some resistance but can be separated by hand

- grade 2; adhesions that require blunt instrument dissection to

10   separate - grade 3; and dense thick adhesions which require sharp

instrument dissection in the plane of the adhesion to separate -

grade 4.

Rat cecum model results

The control group without treatment shows consistently

15   dense and extensive adhesions. The extent of abraded area

covered with adhesions was seen to be 73±21% (mean ± S.D., n=7).

The severity of adhesions was grade 3.5±0.4. Most of the

adhesions were dense and fibrous, involving the cecum with

itself, with the peritoneal wall and with other organs such as

20   the liver, small intestine, and large intestine. Frequently the

nesentery was seen to be involved in adhesions. In the control

group with the application of precursor solution but without

gelation by exposure to the LWUV lamp, the extent of adhesion was

60 ± 24% (n=7), and the severity of adhesions was 3.1 ± 0.4. In

25   the gel treated group, the cecum was seen to be completely free

of adhesions in 6 out of 7 animals. In one case, a grade 2

adhesion was seen with the mesentery over 10% of the area and a

grade 2.5 adhesion was seen over 15% of the area, bridging the

cecum to the sutures on the site of the incision in the

5   peritoneal wall. The overall adhesion extent for the group was

4%, and the overall severity was 0.32. No evidence of residual

gel was visible, the gel presumably having degraded within the

prior two weeks. The cecum appeared whitish with a fibrous layer

on the surface in the control group, but the tissue appeared

10   healthy and normal in animals treated with the gel.

Rabbit uterine horn model

Eight sexually mature female New Zealand rabbits

between 2 and 3 kg in weight were prepared for surgery. A

midline incision was made in the lower abdominal region under

15   Rompun, Ketamine, and Acepromazine anesthesia. The uterine horns

were located and the vasculature to both horns was systematically

cauterized to induce an ischemic injury. One animal was rejected

from the study due to immature uterine horns. Seven rabbits were

selected for the treatment with only the photopolymerizable

20   hydrogel and two animals were selected for evaluating the

combined efficacy of the hydrogel with a fibrinolytic agent,

tissue plasminogen activator (tPA). 5 mg of tPA/ml macromer

solution was used in the latter case. After cauterization,

macromer solutions (0.5 ml) were applied along the horn and

25   allowed to coat the surface where the cauterization injury had

been induced. After uniform application of the solution was

complete, the horns were exposed to a LWUV lamp for 1 min to

induce gelation. The procedure was repeated on the reverse side

of the horns. The incisions were then closed using a continuous

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5   2-0 Vicryl (Ethicon) suture for the musculoperitoneal layer and a

0 Vicryl (Ethicon) suture for the cutaneous layer. No

prophylactic antibiotics were administered. No postoperative

complications or infections were observed. Five animals were

used in the control group. The ischemic injury was made as

10   described and the incision was closed without the application of

the precursor; all techniques were identical between the

treatment group and the control group.

Controls were used where the same animal model was

subjected to surgery without application of the macromer; all

15   surgical techniques were identical between the treatment group

and the historical controls.

The rabbits were reoperated under Ketamine anesthesia

at the end of two weeks to evaluate adhesion formation; they were

sacrificed by introcardiac KCl injection. Adhesion formation was

20   evaluated for extent and tenacity. Extent of adhesion formation

was evaluated by measuring the length of the uterine horn that

formed adhesions with itself or with the peritoneal wall or other

organs. Tenacity of adhesion was classified as either filmy or

fibrous. Filmy adhesions were usually transparent, less strong,

25   and could be freed by hand. The fibrous adhesions were dense,

whitish, and usually required sharp instrument dissection to be

freed. In cases where only a single filmy adhesion band was

evident, a score of 5% was assigned.

5   Typical samples of the horn were excised for histology

and were fixed in a 10% neutral buffered formalin solution.

Paraffin sections of the samples were stained using hematoxylin

and eosin.

         Rabbit uterine horn model results

10   The adhesion score is the % of affected area occupied

by the adhesions, with grading of each as being filmy or fibrous.

Distorted horn anatomies were observed in control animals. The

mean score in the control group was 50 ± 15% of the affected area

of the horn being occupied by adhesions with 10% of these being

15 filmy and 90% fibrous. Distorted horn anatomies were observed,

as can be seen from Figure 19A which presents a superior view of

the uterine horn in an animal used as a control, which showed

adhesions over 66% of the horn surface. The group of animals

treated only with the photopolymerized macromer showed an

20 adhesion score of 13±11.4% (n=10). Of these, 4 animals showed

less than 5% adhesions with only an occasional filmy band

visible.

         The animals treated with photopolymerized gel

containing tPA showed further improved results over the "gel

25 only" animals. One animals showed a filmy band on both the right

and left horn. They were assigned a score of 5% with a total

score of 10%. The other animal did not show any adhesions at

all. Thus the total score for these animals was 5±5%.

5   Figure 19B shows normal horn anatomy in a typical horn

which has undergone gel treatment. Adhesions are filmy in all

cases and no dense bands are seen. No traces of the remaining

gel could be observed. Typical samples of horns showing filmy

adhesions showed some fibrous tissue with a 6-15 cell thick layer

10   of fibroblasts showing some collagen fibrils but no formation of

dense collagen fibers. The horns showing no adhesions

occasionally showed a 1-4 cell thick layer of fibroblasts, but

mostly a normal epithelium with no evidence of inflammatory

cells.

15   This same procedure was slightly modified as described

below as a better mode of using the polymers to prevent

postoperative adhesions using the rat uterine horn model.

          Female rats were anesthetized with pentobarbital (50

mg/kg, intraperitoneally), and a midline laparotomy was

20   performed. The uterine horns were exposed, and the vasculature

in the arcade feeding the horns was systematically cauterized

using bipolar cautery; the most proximal and most distal large

vessel on each horn were not cauterized. Following this, the

antimesenteric surface of each horn was cauterized at two 1 mm

25   diameter spots on each horn, each separated by a 2 cm distance,

the pair centered along the length of each horn. Following

injury, 0.5 ml of macromer solution was applied per horn and was

gelled by exposure to long wavelength ultraviolet light (365 nm,

approximately 20 mW/cm^2) for 15 sec per surface on the front side

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5   and on the back side each. The uterus was replaced in the

peritoneal cavity, and the musculoperitoneal and skin layers were

closed.

The macromer consisted of a PEG chain of MW 8,000

daltons, extended on both sides with a lactic acid oligomer of an

10   average degree of polymerization of 5 lactidyl groups, and

further acrylated nominally at both ends by reaction with

acryloyl chloride. In one batch, Batch A, the degree of

acrylation was determined by NMR to be approximately 75%, and in

another, Batch B, it was determined to be greater than

15   approximately 95%. The macromer was dissolved in saline at a

specified concentration, and the initiation system used was 2,2-

dimethoxy-2-phenyl acetophenone from a stock solution in N-vinyl

pyrrolidinone, the final concentration of 2,2-dimethoxy-2-phenyl

acetophenone being 900 ppm and the final concentration of N-vinyl

20   pyrrolidinone being 0.15%.

In one set of experiments, macromer from Batch A was

applied in varying concentrations, and adhesions were scored at 7

days postoperatively. Scoring was performed by two means. The

length of the horns involved in adhesions was measured with a

25   ruler, and the fraction of the total length was calculated. The

nature of the adhesions was also scored on a subjective scale, 0

being no adhesions, 1 being filmy adhesions that are easily

separated by hand, and 2 being dense adhesions that can only be

separated by sharp instrument dissection. Furthermore, one of

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5   the samples contained tissue-plasminogen activator (t-PA), which

is known to reduce adhesions, at a concentration of 0.5 mg/ml

(0.5%) macromer solution.   The results are shown in Table 21 for

macromer batch A and batch B.

        In a third set of experiments, adhesions were formed in

10   female rats as described above, and the adhesions were surgically

lysed 7 days after the initial surgery.   The extent and grade of

adhesions was scored during lysis.   The animals were divided into

two groups, and one group was treated with macromer from Batch B

at a concentration of 10%.   The results are shown in Table 21 as

15   batch B, 10%.

5 **Table 21: Reduction of Adhesions with Polymer.**

Concentration adhesions	Extent of adhesions % (S.D.)	Grade of adhesions (0-2)	Number of Animals	macromer
Polymer A				
15%	24.6 (3.1)	1.1 (0.1)	7	
20%	33.6 (9.8)	1.2 (0.3)	7	
25%	37.5 (11.1)	1.2 (0.1)	7	
30%	54.2 (12.0)	1.6 (0.4)	6	
20% + t-PA	18.3 (6.4)	1.1 (0.1)	6	
Control (saline)	72.6 (18.7)	1.5 (0.2)	7	
Polymer B				
5%	22.1 (4.2)	1.2 (0.1)	7	
10%	10.0 (5.1)	1.0 (0)	7	
15%	17.8 (5.7)	1.0 (0)	7	
20%	26.3 (11.4)	1.4 (0.2)	7	
Control (saline)	75.9 (4.4)	1.8 (0.3)	7	

Polymer B, 10%

Scoring performed at:	group that became:			
time of lysis	Controls	85.9 (9.7)	1.8 (0.1)	7
Time of lysis	Treatment	79.4 (6.8)	1.7 (0.2)	7
7 days post-lysis	Controls	78.8 (11.3)	1.8 (0.1)	7
7 days post-lysis	Treatment	28.2 (5.1)	1.0 (0)	7

The above results illustrate that the photopolymerized macromer can reduce or prevent post operative adhesions in both primary adhesions and adhesiolysis models, and moreover that the gel can be used to locally release a drug to exert a combined beneficial effect.

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Example 45

Nerve anastomosis

The sciatic nerve of a rat was aseptically severed using a scalpel and allowed to pull apart. The two ends of the nerve were reopposed using sterile forceps, and a 50% solution in buffer of polymer 1KL, a macromer made from PEG 1K with lactide chain extension and acrylate termination, with 0.1% 2,2-dimethoxy-2-phenoxy acetophenone was applied to the nerve stumps. The affected area was illuminated with a 100 W LWUV lamp for 60 seconds, and an adhesive bond was observed to form between the proximal and distal nerve stumps.

To ensure the biocompatibility of the applied material with the nerve tissue, the same solution of macromer was applied to nonsevered rat sciatic nerves, and the area of the incision was closed using standard small animal surgical technique. The area was reopened at 1 hour or 24 hour postoperatively, and the affected area of the nerve was removed en block and prepared for transmission electron microscopy. No morphological differences were observable between the treated nerves at either time point as compared to control rat sciatic nerves that were otherwise nonmanipulated, even though they had been traumatized and manipulated.

5   **Example 46**

Evaluation of PEG Based Degradable Gels as Tissue Adhesives

Abdominal muscle flaps from female New Zealand white
rabbits were excised and cut into strips 1 cm X 5 cm. The flaps
were approximately 0.5 to 0.8 cm thick. A lap joint, 1 cm X 1
10   cm, was made using two such flaps. Two different compositions,
0.6KL and 1 KL, were evaluated on these tissues. Both these
compositions were viscous liquids and were used without further
dilution. 125 μl of ethyl eosin solution in N-vinyl pyrrolidone
(20 mg/ml) along with 50 μl of triethanolamine was added to each
15   ml of the adhesive solution. 100 μl of adhesive solution was
applied to each of the overlapping flaps. The lap joint was then
irradiated by scanning with a 2 W argon ion laser for 30 sec from
each side. The strength of the resulting joints was evaluated by
measuring the force required to shear the lap joint. One end of
20   the lap joint was clamped and an increasing load was applied to
the other end, while holding the joint was clamped and an
increasing load was applied to the other end, while holding the
joint horizontally until it failed. Four joints were tested for
each composition. The 1KL joints had a strength of 6.6±1.0 KPa
25   (mean ±S.D.), while the 0.6KL joints had a strength of
11.4±2.9KPa. It is significant to note that it was possible to
achieve photopolymerization and reasonable joint strength despite
the 6-8 mm thickness of tissue. A spectrophotometric estimate

5   using 514 nm light showed less than 1% transmission through such

muscle tissue.

Example 47

Coupling of Photopolymerizable Groups to Proteins (Albumin)

PEG (M.W. 2,000) monoacrylate (5g) was dissolved in 20

10   ml dichloromethane. Triethyl amine (0.523 g) and 2,2,2-

trifluoroethanesulfonyl chloride (tresyl chloride) (0.017 g) were

added and the reaction was allowed to proceed for 3 hours at 0°C

under nitrogen atmosphere. The reaction mixture was then

filtered and the dichloromethane evaporated to dryness. The

15   residue was redissolved in a small amount of dichloromethane and

precipitated in diethyl ether. The polymer was then filtered and

dried under vacuum for 10 hours and used directly in the

subsequent reaction with albumin.

1 g of bovine serum albumin was dissolved in 200 ml of

20   sodium bicarbonate buffer at pH 9. Tresyl activated PEG

monoacrylate (5 g) was added and the reaction was stirred for 24

hours at 25°C. Albumin was separated by pouring the reaction

mixture into acetone. It was further purified by dialysis using

a 15,000 daltons cutoff dialysis membrane. A 10% w/v solution of

25   the PEG acrylated albumin could be photopolymerized with long

wave UV radiation using 0.9 mg/ml of 2,2 dimethoxy 2

phenylacetophenone as the initiator. In this gel the degradable

segment is the protein albumin.

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5   **Example 48**

Modification of Polysaccharides (Hyaluronic Acid)

In a dry 250 ml round bottom flask, 10 grams of PEG 400

monomethacrylate was dissolved in 100 ml dry dioxane, to which

4.053 g of carbonyl diimidazole (CDI) was slowly introduced under

10   nitrogen atmosphere and the flask was heated to 50°C for 6 h.

Thereafter the solvent was evaporated under vacuum and the CDI

activated PEG monomer was purified by dissolving in

dichloromethane and precipitating in ether twice.

1 g of hyaluronic acid, 5 g of CDI activated PEG 400

15   monoacrylate were dissolved in 200 ml sodium borate buffer (pH

8.5) and the solution was stirred for 24 hours. It was then

dialyzed using a 15,000 dalton cutoff dialysis membrane to remove

unreacted PEG. A 10% w/v solution of the acrylated hyaluronic

acid was photopolymerized with long wave UV radiation, using 0.9

20   mg/ml of 2,2-dimethoxy-2-phenylacetophenone as the initiator. In

this gel, the degradable region is hyaluronic acid.

                               Example 49

PEG Chain Extended with Polyorthocarbonates and Capped

With Urethane Methacrylate

25   3, 9-bis(methylene) 2,4,8,10-tetraoxaspiro [5,5]

undecane (1g) and polyethylene glycol (molecular weight, 1,000,

7.059 g) were weighed into a 250 ml Schlenk tube under dry

nitrogen atmosphere in a glove bag. 50 ml of dry tetrahydrofuran

                               - 130 -

5   was introduced under nitrogen atmosphere and reaction mixture was

stirred for 6 hours at 50°C. This is a typical step growth

reaction with a disturbed stoichiometry, resulting in low

molecular weight poloyorthocarbonate with terminal hydroxy

groups. The oligomer was separated by precipitating in hexane

10   and dried under vacuum. 5 g of oligomer was redissolved in dry

THF to which 20 μl of dibutyltindilaurate and 2 ml of 2-

isocyanatoethyl methacrylate were slowly introduced and

temperature was raised to 50°C. It was held there for 6 hours

and cooled. The product was separated by precipitation in

15   hexane. In this gel, the degradable region is a

polyorthocarbonate.

Example 50

Microencapsulation of Animal Cells

       A 23% w/w solution of 18.5KG in HEPES buffered saline

20   (5 ml) was used to resuspend 10^6 CEM-SS cells. Ethyl eosin (10^{-4}

M) was used as a solution in N-vinyl pyrrolidone as the initiator

and triethanolamine (0.01 M) was used as the coinitiator. The

solution was then exposed through a coextrusion apparatus to an

argon ion laser (514 nm, 2 Watts). The coextrusion apparatus had

25   mineral oil as the fluid flowing annularly (flow rate 4 ml/min)

around an extruding stream of the precursor cell suspension (flow

rate 0.5 ml/min). The microdriplets gelled rapidly on being

exposed to the laser light and were collected in a container

5   containing PBS. The oil separated from the aqueous phase and the

microspheres could be collected in the PBS below. The

microspheres formed were thoroughly washed with PBS buffer to

remove unreacted monomer and residual initiator. The size and

shape of microspheres was dependent on extrusion rate and

10 extruding capillary diameter (18 Ga to 25 Ga). The

polymerization times were dependent on initiator concentration

(ethyl eosin 5 μM to 0.5 mM, vinyl pyrrolidone (0.001% to 0.1%),

and triethanolamine (5 mM to 0.1 M), laser power (120 mW to 2W),

and monomer concentration (>10%w/v). Spheres prepared using this

15 method had a diameter from 500 μm to 1,200 μm. The

polymerizations were carried out at physiological pH in the

presence of air. This is significant since radical

polymerizations may be affected by the presence of oxygen. Cell

viability subsequent to encapsulation was checked by trypan blue

20 exclusion assay and the encapsulated cells were found to be more

than 95% viable after encapsulation.

Example 51

Various Formulations for the Prevention of

Post Operative Adhesions

25   The utility of PEG-oligo(α-hydroxy acid) diacrylates

and tetraacrylates to prevent postoperative adhesions was

evaluated in the rabbit uterine horn model as described above.

The following polymers were synthesized, as described above: PEG

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5   6K lactide diacrylate (6KL), PEG 10K lactide diacrylate (10KL).

PEG 18.5K lactide (18.5KL), PEG 20K lactide (20KL). Solutions

with 24% polymer in PBS with 900 ppm 2,2-dimethoxy-2-phenyl

acetophenone, were prepared as described above. The solutions

were applied to the uterine horn after cautery of the vascular

10   arcade and illuminated with a 365 nm LWUV lamp, as described

above. In one formulation, 18.5KL, 5 mg t-PA was mixed into the

solution before application. Controls consisted of animals

manipulated and cauterized but not treated with macromer

solution. Measurement was performed on the 14th ± 1 day. Extent

15   of adhesion was estimated from the fraction of the horn that was

involved in adhesions, and the tenacity of adhesions was scored

as 0, no adhesions; 1, filmy adhesions that offer no resistance

to dissection; 2, fibrous adhesions that are dissectable by hand;

3, fibrous adhesions that are dissectable by blunt instruments;

20   and 4, fibrous adhesions that are dissectable by sharp

instruments. The results were as follows, where the extent of

adhesions and the tenacity of the adhesions are shown.

Table 22: Efficacy of Polymer in Preventing Adhesions.

Formulation	Number of animals	Extent, %, ± S.D.	Tenacity, 0-4 ± S.D.
6KL	7	0.9 ± 1.7	0.9 ± 0.7
10KL	7	0 ± 0	0 ± 0
20KL	6	4.4 ± 5.0	0.9 ± 0.7
18.5KL	7	8.9 ± 13.1	1.6 ± 1.3
t-PA Control	7	35 ± 22	3.3 ± 0.6

Example 52

Polymerization of Ultrathin Layers of Polymer on the Surface of Blood Vessels to Reduce Thrombosis After Vessel Injury

Blood vessels were harvested from rats and were rinsed free of blood. The endothelium of the vessel were removed by inserting a wooden dowel and rotating the vessel over the dowel. One vessel was used as a control, and was exposed to flowing blood as described below without further modification. Another vessel was treated first by exposure to eosin Y at 1 mM in saline, then rinsed in HEPES buffered saline, then filled with a solution of PEG-MA, PEG 10K with acrylate end-capped oligomers of DL lactide, containing triethanolamine (TEA) (100 mM) and N-vinylpyrrolidone (VP) (0.15%) and then illuminated by exposure to an argon ion laser at 0.5 W/cm2 for 15 sec. The nonpolymerized prepolymer mixture in the lumen of the vessel was rinsed away with saline. Human blood was collected from the antecubital vein and was anticoagulated with heparin at 2 units/ml. This blood

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5   was perfused through each vessel by a syringe pump at a flow rate

corresponding to a wall shear rate of approximately 200/s for 7

min. The vessel was then superficially rinsed in saline and

fixed in formaldehyde.

The treated vessel did not appear colored or different

10   in color after perfusion compared to its color before perfusion,

while the untreated control vessel appeared blood red. Thin

segments of each vessel were cut from each vessel, were mounted

on end, and were examined by environmental scanning electron

microscopy (ESEM). ESEM is performed on hydrated samples in

15   relatively low vacuum. This permits the visualization of the

polymer film coating in the swollen and wet state. This is

important to obtain measurements that may be readily interpreted,

since the polymer film is approximately 95% water. A high degree

of thrombosis was readily observed in the control vessel. The

20   lumen of this vessel was narrowed to less than one-third its

diameter pre-perfusion by the accumulation of thrombus, as shown

in Figure 20A. By contrast, no thrombus could be observed in the

lumen of the treated vessel, as shown in Figure 20B. A higher

magnification of the vessel wall demonstrated no adherent

25   thrombus. A still higher magnification shows a white structure

which is the polymer film, which is different in contrast from

the tissue due to differential charging under the electron beam

of the ESEM. The film may be seen to be precisely conformed to

the shape of the vessel and be approximately 5 - 8 μm thick.

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5   The region of polymerization was restricted to the
neighborhood of the blood vessel wall surface. The
photosensitive dye was adsorbed to the vessel wall. Unbound dye
was rinsed away. The entire lumen was filled with prepolymer,
but upon illumination the gel formation was restricted to the
10   vessel wall where the dye and the prepolymer meet. This
interfacial polymerization process can be conducted to produce
surface adherent layers that vary in thickness from less than 7
μm to more than 500 μm.

        The above procedure was performed in 8 control rat
15   arteries, and 8 treated arteries, with equivalent light
microscopic histological results as described above. As
demonstrated by this study, PEG prepolymers can be polymerized
upon the lumenal surface of blood vessels. The immediate effect
of this modification is to reduce the thrombogenicity of an
20   injured blood vessel surface. This has clear utility in
improving the outcome of balloon angioplasty by reducing the
thrombogenicity of the vessel and lesion injured by balloon
dilation. Another effect of this modification is to be reduce
smooth muscle cell hyperplasia. This may be expected for two
25   reasons. First, platelets contain a potent growth factor,
platelet-derived growth factor (PDGF), thought to be involved in
post-angioplasty hyperplasia. The interruption of the delivery
of PDGF itself poses a pharmacological intervention, in that a
"drug" that would have been delivered by the platelets would be

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5   prevented from being delivered. Thrombosis results in the

generation of thrombin, which is a known smooth muscle cell

mitogen. The interruption of thrombin generation and delivery to

the vessel wall also poses a pharmacological intervention. There

are other growth factors soluble in plasma which are known to be

10   smooth muscle cell mitogens. The interruption of thrombin

generation and delivery to the vessel wall also poses a

pharmacological intervention. Moreover, there are other growth

factors soluble in plasma which are known to be smooth muscle

cell mitogens. The gel layer is known to present a permselective

15   barrier on the surface of the tissue, and thus the gel layer may

reasonably be expected to reduce hyperplasia after angioplasty.

The inhibition of thrombosis upon the vessel wall may also reduce

the incidence of abrupt reclosure and vasospasm, both of which

occur sometimes following vascular intervention.

20   **Example 53**

Interfacial Polymerization of Macromers

Inside Blood Vessels to Prevent Thrombosis

Macromer solutions were polymerized interfacially

within previously injured blood vessels *in vivo* to prevent

25   thrombosis. The carotid artery was exposed, and a polyethylene

tube (PE-10) was used to cannulate the exterior carotid artery.

The artery was clamped with fine arterial clamps proximal to the

interior/exterior carotid artery bifurcation and approximately 2

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5   cm distal to the bifurcation. A 1 ml tuberculin syringe was used

to rinse the blood from the lumen of the isolated zone by filling

and emptying the vessel zone. The vessel was injured by crushing

using a hemostat. The isolated zone was filled with a 10 mM

solution of eosin Y for 2 minutes, after which it was rinsed and

10   filled with a 20% solution of a macromer in saline with 0.1 mM

triethanolamine and 0.15% N-vinyl pyrrolidinone. The macromer

consisted of a PEG chain of MW 8,000 daltons, extended on both

sides with a lactic acid oligomer of an average degree of

polymerization of 5 lactidyl groups, and further acrylated

15   nominally at both ends by reaction with acryloyl chloride. The

vessel was illuminated transmurally using an argon ion laser (514

nm) at an intensity of approximately 1 mW/cm^2 for 5 seconds.

Following this, the cannula was removed from the exterior carotid

artery and the artery was ligated at the bifurcation. The

20   arterial clamps were removed to permit the resumption of blood

flow. Perfusion was allowed for 20 minutes, following which the

vessel were again isolated, removed from the body, gently rinsed,

fixed, and prepared for light microscopic histological analysis.

Using the naked eye, the crushed segments in control animals,

25   which lacked illumination, were red, indicating internal thrombus

with entrapped red blood cells. By contrast, no redness was

observed at the site of the crush injury in the treated vessels.

Histology showed extensive thrombus, fibrin, and entrapped red

blood cells in the non-treated vessels. By contrast, no thrombus

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5   or fibrin or entrapped red blood cells were observed in the

       treated vessels.   The procedure was conducted in four control

       animals and three treated animals.

              This example demonstrates that the polymerization can

       be carried out *in situ* in the living animal, that the polymer

10   coating remains adherent to the vessel wall during arterial blood

       flow, and that the polymer coating can prevent thrombosis *in vivo*

       in non-anticoagulated animals.   This approach to treatment has

       clear benefits in preventing abrupt reclosure, vasospasm, and

       restenosis after intravascular interventional procedures.

15   Moreover, it is more generally applicable to other intraluminal

       and open-surface organs to be treated.

9.   The crosslinked biocompatible material of claim 8, wherein the biologically active materials are living cells selected from islet of Langerhans, dopamine secreting cells, crythropoietin secreting cells, nerve growth factor secreting cells, parathyroid cells, or norepinephrine/-metacephalin secreting cells.

10.   The crosslinked biocompatible material of claim 8, wherein the biologically active material is a drug

11.   A crosslinked biocompatible material comprising: at least one ionically crosslinked component; and at least one covalently crosslinked component is derived from a polyalkylene oxide.

12.   The crosslinked biocompatible material of claim 11, wherein the covalently crosslinked component is polyethylene glycol diacrylate.

13.   The crosslinked biocompatible material of claim 11, further comprising a biologic encapsulated by the material.

14.   The crosslinked biocompatible material of claim 13, wherein the material is effective to provide immunoprotection for the biologic in a physiological environment.

15.   The crosslinked biocompatible material of claim 14, wherein the material provides immunoprotection of the biologic when xenotransplanted.